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# A STUDY OF THE CHROMOSOME NUMBER IN SOME INDIAN MEMBERS OF THE FAMILY *CODONIACEAE*.

BY P. N. MEHRA.

(From the Kashyap Research Laboratory, Punjab University, Lahore.)

Received May 14, 1938.

[Communicated by Dr. H. Chaudhuri, D.Sc. (Lond.), Ph.D., D.I.C.]

THREE closely allied members of the family Codoniaceæ, *Fossombronia himalayensis* Kash., *Petalophyllum indicum* Kash., and *Sewardiella tuberifera* Kash. have been studied.

It is very difficult to make successful smears of the spore-mother cells of these species for studying the chromosome numbers. The mother cells are agglutinated together in a mucilaginous substratum formed of the gelatinous walls of the mother cells and consequently cannot be drawn on a slide in a one-cell thick layer. Even the microtome sections of the spore-mother cells do not give good results for two reasons. Firstly, the nucleus is lodged in the central narrow space of the deeply 4-lobed mother cell at the time of meiosis and mitosis and the chromosomes consequently are jumbled. Secondly, the mother cell is packed with food grains and chlorophyll granules which considerably obscure the clarity of the dividing nucleus. The easiest method for studying the chromosome number of these species is to cut microtomic sections 8–10  $\mu$  thick of very young sporogonia where the cells of the archesporial tissue are in an active state of division. Five or more sporogonia are embedded in one block and if the sporogonia are properly fixed definite results are obtained from the sections of one block.

Bouin's Fixative Allen's modification P. E. B.<sub>15</sub> was used in all cases.

*Fossombronia himalayensis* Kash.

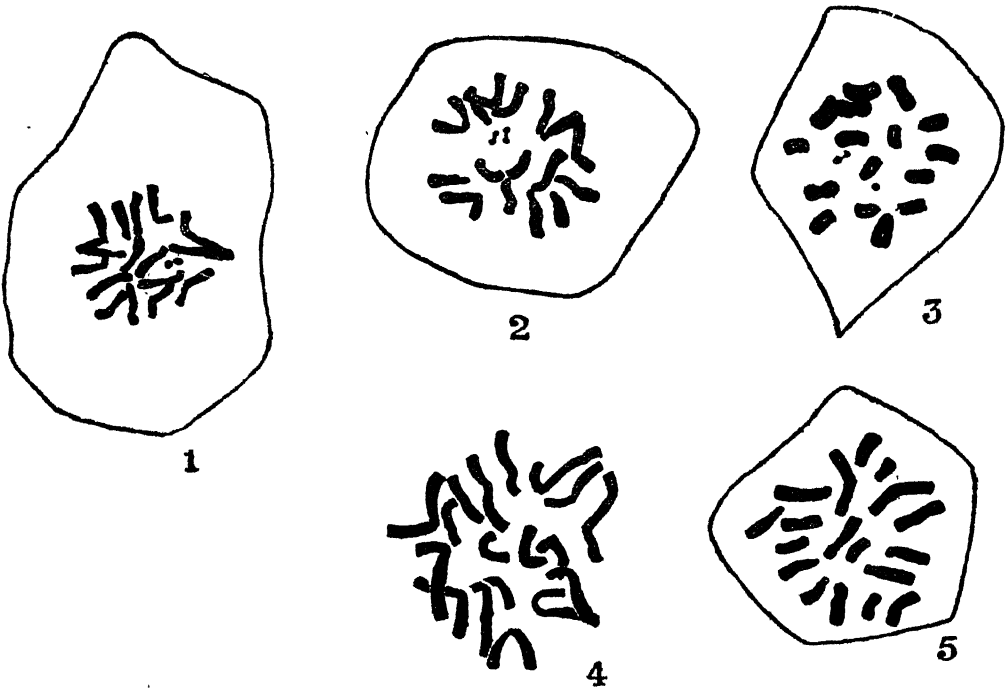
The species was first discovered by Kashyap in 1915,<sup>1</sup> and later in 1917 by Stephani who named it *Fossombronia Levieri*.<sup>4</sup>

The plant is quite common on the outer ranges of the North Western Himalayas between 5000 ft. and 7000 ft. being met with at Simla, Mussoorie, Dalhousie, etc. It has also been reported from South India from Bombay and Nilgiris.<sup>3</sup>

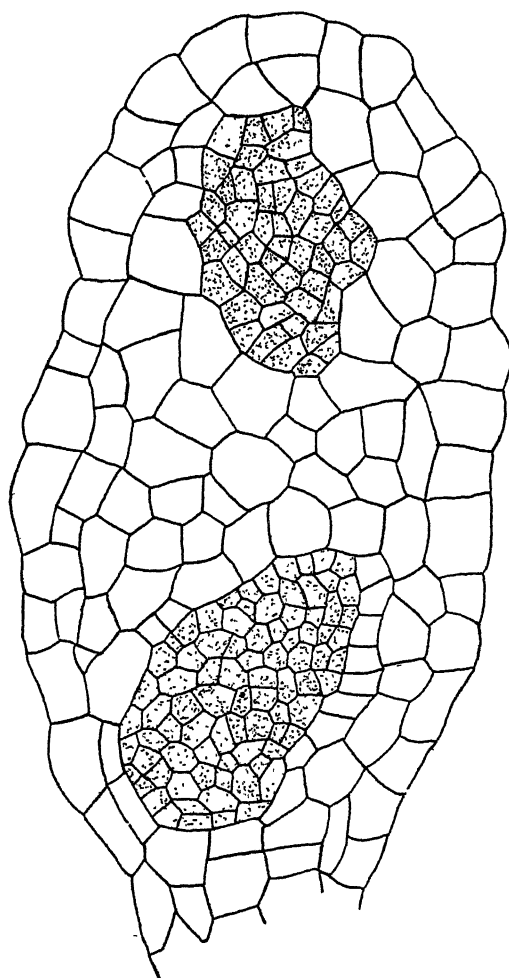
The fixation of the young sporogonia was made twice on 25th and 29th August 1935 at Mussoorie between 3 p.m. and 6 p.m. and the fixative allowed to act for 5-6 hours followed by the usual process of washing, clearing, embedding, etc. Sections were cut 8-10  $\mu$  thick.

In the cells of the archesporium the nuclei are observed at all stages of mitotic division. A number of figures with the chromosomes spread out in the form of a flat plate at the metaphase are observed. The  $2x$  number in the species is 18 beyond doubt (Figs. 1, 2, 3). In the last the splits in the individual chromosomes can be seen.

There is a pair of V-shaped chromosomes with the fibre attachment constriction in the middle, a pair of L-shaped chromosomes and a pair of very small chromosomes which in some figures appear dot-like. The morphology of the other chromosomes cannot be clearly made out because mostly they are alike with slight differences in their size.



An interesting abnormality in a young sporogonium is observed. Normally the archesporium fills the entire cavity of the sporogonium but in one instance sterilisation of the archesporial tissue had occurred in the middle so that the sporogonium was divided into two compartments each possessing its own archesporium (Fig. 6). In another abnormal case the wall of the sporogonium in the upper region was very thick and projected



6

into the interior of its cavity so that the archesporium was in the form of a shallow cup appearing more or less lunar in vertical section.

*Petalophyllum indicum* Kash.

The plant was discovered for the first time by Kashyap in the year 1928<sup>2</sup> from Lahore on the banks of river Ravi and since then it has also been observed on the banks of river Beas as well. It is endemic to the Punjab plain since it is not reported from any other locality.

The plants grow on moist clay, particularly along the edges of pools with standing rain water in shady places. Usually they occur scattered but sometimes in small patches of a few individuals.

Young sporogonia were fixed on 1-11-35 and 3-11-35 between 2 p.m. and 3 p.m. Chromosome counts were made from a number of different sections of the nuclei of the archesporium at the metaphase. It may be said that the  $2x$ -chromosome number is definitely 18 in the sporophyte as in *Fossombronia himalayensis* (Fig. 4). The morphology of chromosomes in the two cases, however, is very different. There is no dot-like pair of chromosomes characteristic of the previous species.

*Sewardiella tuberifera* Kash.

This monotypic genus was discovered by Kashyap in 1915.<sup>1</sup>

The plants occur very commonly at Mussoorie only along Tehri Road and in Simla within a range of 5000-7000 ft. The genus is endemic in the Punjab Himalayas.

The plants grow commonly in thick clusters in shady and humid places sheltered over by ferns, grasses and other herbaceous plants. Not uncommonly they occur singly when they are quite robust.

Young sporogonia were fixed in the first week of September 1935 at Mussoorie.

The  $2x$ -chromosome number in the species is 18 beyond doubt as determined from the division of the archesporial cell nucleus at metaphase (Fig. 5). This number has been verified in a number of very clear sections. The morphology of the chromosomes is different from the previous two species. There is no dot-like pair of chromosomes which is met with in *Fossombronia himalayensis*.

*Conclusion.*

Below is given the chromosome numbers of the members of the family Codoniaceæ so far worked out (compiled from three papers by Tischler).<sup>5, 6, 7</sup> Those marked with asterisk are the result of the present investigation.

<i>Pellia calycina</i>	..	..	9	Heitz 1927, Schowalter 1927.
<i>Pellia epiphylla</i>	..	..	8	Farmer U. Reeves 1894, Farmer 1895, Davis 1901, Chamberlain 1903.
			9	Heitz 1927, Wolfson 1927, Lorbeer 1934.
<i>Pellia epiphylla</i> var. <i>bivalens</i>	..		18	Heitz 1927.

<i>Pellia Fabbriana</i>	..	..	9	Heitz 1927, 1928, Tatuno 1933, 1934, Lorbeer 1934.
<i>Pellia Neesiana</i>	..	..	9	Heitz 1927, 1928, 1931, Schowalter 1928, Tatuno 1933, 1934, Lorbeer 1934.
<i>Pellia translucida</i>	..	..	9	
<i>Pellia borealis</i>	..	..	18	Lorbeer 1934.
<i>Blasia pusilla</i>	..	..	5-6	Woodburn 1913.
			9	Heitz 1927, 1928, Lorbeer 1934.
<i>Treubia insignis</i>	..	..	8	Grün 1913.
			9	Lorbeer 1934.
<i>Makinoa crispata</i>	..	..	9	Heitz 1927, 1928, Sinoto 1930, Yazawa 1931, Tatuno 1933, 1934, Lorbeer 1934.
<i>Androcryphia confluens</i>	..	..	9	Heitz 1937, 1928.
<i>Androcryphia confluens</i> var. <i>bivalens</i>			18	Heitz 1928.
<i>Calycularia radiculosa</i>	..	..	8	Campbell 1913.
<i>Fossombronina Dumortieri</i>	..	..	8	Farmer 1895.
<i>Fossombronina longiseta</i>	..	..	8	H. B. Humphrey 1906.
<i>Fossombronina cristata</i>	..	..	ca 4	Haupt 1920.
<i>Fossombronina angulosa</i>	..	..	9	Lorbeer 1934.
<i>Fossombronina caespitiformis</i>	..	..	9	Heitz 1927, Lorbeer 1934.
<i>Fossombronina Husnoti</i>	..	..	(8-) 9	Heitz 1927.
<i>Fossombronina pusilla</i>	..	..	8	Chaloud 1930.
* <i>Fossombronina himalayensis</i>	..	2x = 18		Mehra 1938.
<i>Petalophyllum Ralfsii</i>	..	..	9	Heitz 1927, Lorbeer 1934.
* <i>Petalophyllum indicum</i>	..	..	2x = 18	Mehra 1938.
* <i>Sewardiella tuberifera</i>	..	..	2x = 18	Mehra 1938.

A glance at the above shows that out of the 24 members so far investigated 19 show the  $x$ -chromosome number to be 9 or a multiple of 9.

The species differing in this respect are :—

<i>Calycularia radiculosa</i>	..	8
<i>Fossombronia Dumortieri</i>	..	8
<i>Fossombronia longiseta</i>	..	8
<i>Fossombronia cristata</i>	..	ca 4
<i>Fossombronia pusilla</i>	..	8

It is well known that owing to the small size of the chromosomes, unless a critical study is made and the counts verified in a number of different preparations there is a possibility of some error, particularly when some of the chromosomes sometimes are dot-like, like those met with in *Fossombronia himalayensis* and likely to be overlooked. Thus while the previous authors considered the chromosome number in *Pellia epiphylla* to be 8, recent investigations independently by Heitz, Wolfson and Lorbeer point out the number to be 9. In the case of *Blasia pusilla* Woodburn was not certain of the number mentioning it as 5-6 while Heitz and also Lorbeer have shown independently the chromosome number to be 9. Again in *Treubia insignis* while Grün found 8 chromosomes Lorbeer finds the number to be 9.

The fact that the chromosome number in the majority of species is 9 or a multiple of 9 seems to suggest that the basic number for the family Codiaceae is 9. It would be very desirable therefore to re-investigate the species differing in this respect.

It may be pointed out that even in the same genus *Fossombronia* different chromosome numbers have been reported. Out of the 7 species worked out, 3 are reported to possess 8, in one the number is supposed to be probably 4, while in the other 3 including the Indian *F. himalayensis* the number is 9. While it is certainly not impossible that the number may vary, it seems more probable that there will be the same basic number 9 in all the species.

Polyploidy is of rather rare occurrence in the evolution of species in the family. There are only three instances. In *Pellia epiphylla* diploidy has occurred resulting in the formation of a new variety while in *Pellia borealis* double the basic number is met with. Also diploidy has taken place in *Androcryphia confluens* resulting in a new variety.

The strong morphological affinity of the three Indian species *Fossombronia himalayensis*, *Petalophyllum indicum* and *Sewardiella tuberifera* is further strengthened by their possessing the same diploid chromosome number 18 but each differs from the other in possessing different morphology of the chromosomes.

### Summary.

1. The chromosome numbers in three Indian species *Fossombronina himalayensis* Kash., *Petalophyllum indicum* Kash. and *Sewardiella tuberifera* Kash. have been investigated. The diploid chromosome number in each case is 18 but the morphology of the chromosomes in each species is different from the other.

2. The basic chromosome number in the family *Codoniaceæ* appears to be 9.

3. Polyploidy is of rather rare occurrence in the evolution of new species in the family.

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3. ————— .. .. *Liverworts of the Western Himalayas and the Punjab Plain*, 1932, Part II.
4. Stephani, F. .. .. *Species Hepaticarum*, 1917, 6, 74.
5. Tischler, G. .. .. *Tabulæ Biologicæ*, 1927, Band IV.
6. ————— .. .. *Tabulæ Biologicæ, Periodicæ*, 1931, Band I.
7. ————— .. .. *Ibid.*, 1936, Band V.

### EXPLANATION OF FIGURES.

FIGS. 1, 2, 3.—*Fossombronina himalayensis*. × 2760.

FIG. 4.—*Petalophyllum indicum*. × 2760.

FIG. 5.—*Sewardiella tuberifera*. × 2760.

FIG. 6.—Section of the young sporogonium of *Fossombronina himalayensis*. × 330.

# ABNORMAL SPOROCARPS IN *MARSILEA MINUTA* LINN.

By P. N. MEHRA.

(From the Kashyap Research Laboratory, Punjab University, Lahore.)

Received May 14, 1938.

(Communicated by Dr. H. Chaudhuri, D.Sc. (Lond.), Ph.D., D.I.C.)

DURING my observation of the material of the sporocarps of *Marsilea minuta*, I have come across certain abnormalities which, it is thought worthwhile, should be placed on record.

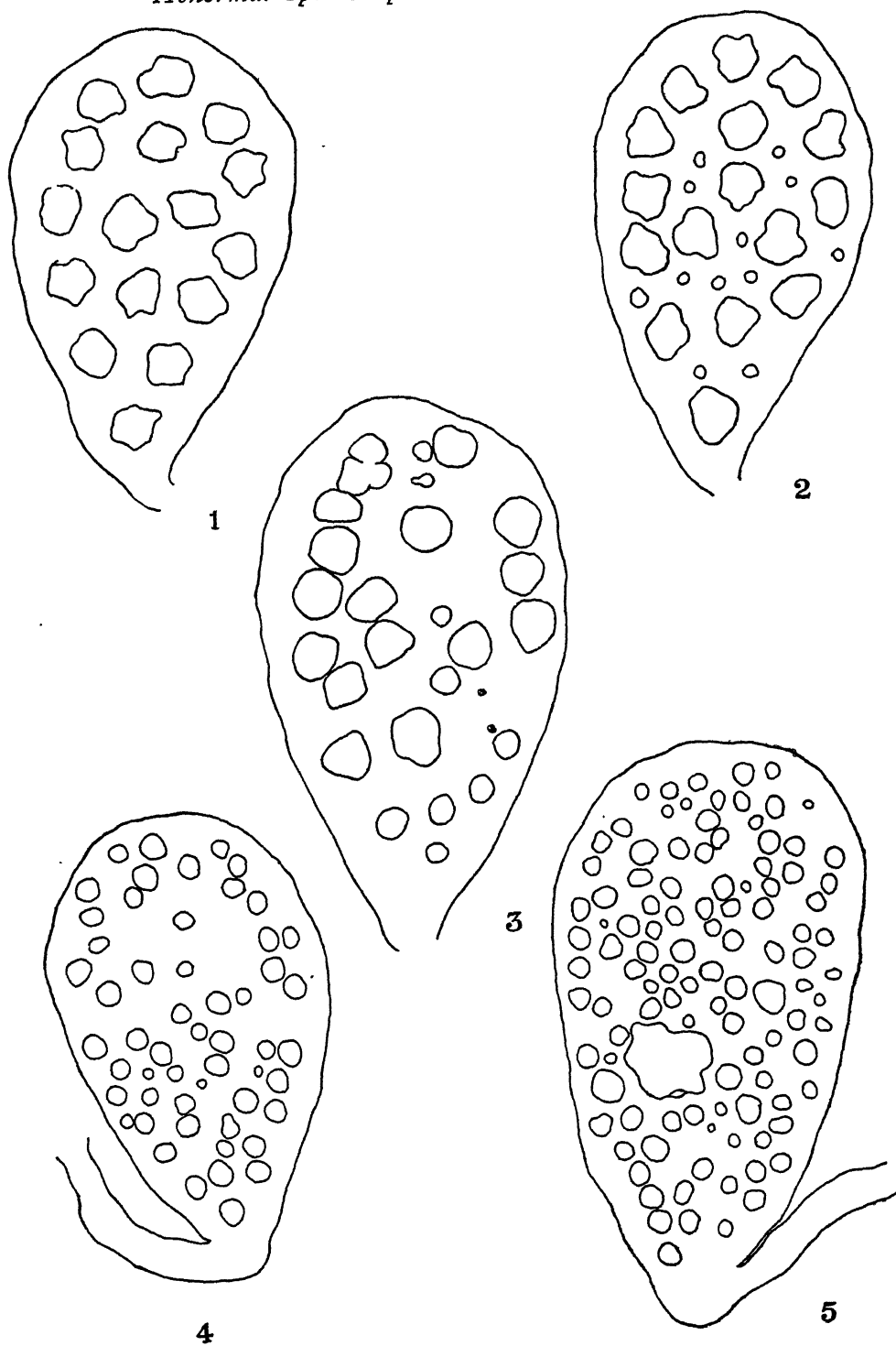
*Marsilea minuta* occurs commonly in the Punjab and is very variable in habit depending upon the conditions of its occurrence. When growing under water it has elongated rhizome with leaves arising at fairly long distances from one another and if the plant is fertile with one or two stalked sporocarps on the adaxial side of the leaf near its base. In rather exposed environments the stem is much condensed and the leaves bearing the sporocarps in their usual position are very much crowded. All sorts of intermediate conditions between the two extremes are met with in intermediate environments. S. S. Pande<sup>1</sup> in his study of *Marsilea minuta* (*M. erose* Willd.) found 64 microspores in a microsporangium and 4-8 megaspores in a megasporangium. This latter condition is different from that described in the megasporangia of other species so far investigated. Following the development of a megasporangium he states, "... walls are laid down round a few nuclei (4-8) and these become megaspores". He, however, failed to germinate these megaspores even on repeated attempts. While it is true that there are 64 microspores in a microsporangium the observation of Pande on the megasporangia is not supported by my observations. In the *normal* material the condition is exactly similar to that described in other species of the genus. In a megasporangium there is a single thick-walled megaspore.

In the *abnormal* material the following interesting conditions are observed.

The megasporangia or microsporangia of the normal type are absolutely lacking in the sporocarp. In the sporangia there are found 16 fairly large irregularly angled spores which apparently seem to be non-functional

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<sup>1</sup> Pande, S. S., "Some Observations on the Biology of *Marsilea*", *Proc. Lahore Philosophical Society*, 4.



(Fig. 1). In these cases the spore-mother cells have been directly transformed into these structures. In other sporangia there are observed 16 such large spores and besides a few other comparatively smaller spore-like bodies with thick walls (Figs. 2, 3). On making observations in a number of such sporangia in which these smaller structures at different stages of development are observed, one is led to believe that probably the nuclei of the tapetal cells when liberated become gradually transformed into these spore-like bodies. In still other cases there may be no large spores formed directly from the mother cells but a number of smaller spore-like structures of different sizes with thick walls. In Fig. 4 about 54 such spores can be counted while in Fig. 5, 107 such spores are observed in addition to a big mass formed either by the fusion of such bodies in their early stages of development or the failure of the parent mass to segregate into these structures.

There is another interesting fact. A plant bears either all the normal type of sporocarps with typical mega- and microsporangia or all the abnormal sporocarps with abnormal sporangia. Never a case when the same plant bears both the normal and the abnormal type of sporocarps has been observed. This fact raises another question. Whether the abnormal sporocarps are borne on plants which may have an abnormal genetic constitution the result of which is the failure in normal meiosis and mitosis or whether they are borne on plants growing in defective environment? I have found the abnormal sporocarps in plants growing in rather dry localities and also in plants submerged under shallow water in which condition they seemed to thrive nicely. In the former case the sporocarps are rather small in size and contain the abnormal sporangia of the type shown in Figs. 1, 2, 3. In the latter, however, the sporocarps externally are of the normal size and form like the typical sporocarps but they contain mostly the abnormal sporangia of the type shown in Figs. 4, 5. In another nearby shallow water pond with environments apparently similar to that where the plants with abnormal sporocarps are found, I have collected plants bearing normal type of sporocarps.

The question as to what determines the formation of abnormal type of sporocarps may yet be considered undecided but the data so far collected seems to suggest the disturbance in the genetic constitution of the plants bearing them as causal factor rather than the effect of the environments. Further work in this connection is in progress.

# HETEROCHROMATIN, SOMATIC "CROSSING-OVER" AND THE INTERCHANGE HYPOTHESIS BETWEEN NON-HOMOLOGOUS CHROMOSOMES.

BY DONTCHO KOSTOFF.

(From the Academy of Sciences; Institute of Genetics, USSR, Moscow.)

Received April 18, 1938.

(Communicated by Prof. C. R. Narayan Rao, M.A.)

THE development of genetics and especially of cytogenetics during the last three decades proceeded so rapidly and the accumulation of differential data in carrying out analytic investigations in these fields of biology was so enormous that it is almost impossible or, better to say, it is very difficult even for a geneticist to keep in contact with all of the trends of the numerous subdivisions in genetics. It is to regret that only a few geneticists work successfully with *Drosophila* and plant objects, while for the great majority of plant geneticists *Drosophila* symbolizings are enigmas at the same time when most of *Drosophila* workers judge the genetic and cytogenetic works carried out with various plants as attempts "to ape the manners of the leaders". The analytic studies in genetics and cytogenetics develop so rapidly that the enormous accumulation of numerous facts in various subdivisions cannot be easily "assimilated" and synthesized. Synthesis is lacking especially between the data accumulated in plant cytogenetics with those of *Drosophila* studies.

It is true that lack of integrity in genetics as in other biological sciences is often the cause for spending an enormous amount of time and energy on experimenting in certain directions without attaining a satisfactory reward. When integrating, new hypotheses and theories emerge which can further stimulate research work.

Presenting here the data of a series of the observations made by the author in correlation with the numerous observations recorded during the last 2-3 decades upon: (1) heterochromatic regions in the chromosomes, (2) somatic crossing-over, (3) variegations (including mosaicism), and (4) the cytogenetic effect of X-rays, radium-rays and temperature treatments, attempts will be made to advance working hypotheses which will help to link numerous cytogenetic phenomena appearing at first sight as they would have nothing in common. A large number of data, most of which are considered here, suggest that certain environmental factors (X-ray, radium,

temperature, etc.) and internal conditions (conditions in the salivary glands, species hybridization, etc.) apparently induce various degrees of chromosome conjugation, and chiasma formation (crossing-over, interchange) sometimes preferentially between the heterochromatic regions of homologous and non-homologous chromosomes. These phenomena are probably responsible in the majority of the cases for the chromosomal interchanges resulting in the experiments and in nature. Chromosome "fragmentations" are results of such interchanges. Direct fragmentations are probably very rare. Variegations (including mosaicism and some types of chimeras) result in the majority of the cases most probably from somatic interchange following conjugation preferentially in heterochromatic regions from homologous or non-homologous chromosomes. Increased somatic "mutation" frequency in hybrids in many cases is an increase of such interchanges. Agents that induce cancer—most probably induce first chromosome conjugation preferentially in the heterochromatic regions and interchanges in soma between homologous and obviously between non-homologous chromosomes. The same conditions seem to exist in certain genotypes. The observations reported by numerous investigators are given down for supporting these main theoretical interpretations and some others advanced in this paper.

The terms heteropyknose, heterochromatin and chromocenter are known for a long time in the cytological literature, but their possible genetic significance was only recently revealed by the pioneer work of Heitz (1928-35) and *Drosophila* cytologists (Painter and Stone, 1935; Frolova, 1936, Prokofieva, 1935, 1937; Kaufmann, 1937, etc.).

The cytologists of the last century and the beginning of the present century have often observed in the nuclei numerous bodies which gave usually microchemical reactions like the basic chromatin. Flemming (1882) called them "Netzknöten", Auerbach (1890)—"Cyanophile Nucleolen", Rosen (1892)—"Pseudonucleolen", Zacharias (1895)—"Nebennucleolen", Zimmermann (1896)—"Chromatinkugeln", Tellyesnicki (1904)—"Nucleosomen", Rosenberg— (1904-09)—"Prochromosomen", Baccarini (1908)—"Chromocenters", Lundegårdh (1910)—"Karyosomen", etc. (cf. Tischler, 1934). Rosenberg's data are of importance. He found that the number of the prochromosomes corresponds to the chromosome number of the plant studied. Rosenberg's statements were confirmed by numerous investigators in studying the pro-chromosomes (chromocenters) of many plants (Overton, 1905, 1909; Myake, 1905; Yamanouchi, 1906; Laibach, 1907; Malte, 1908, 1910; Guttenberg, 1909; Stout, 1913; Schussning and

Olde, 1927 ; Schiller, 1928 ; Kuhn, 1929 ; Geitler, 1928 ; de Souza Violante, 1929 ; Heitz, 1929 ; Grégoire, 1932 ; Janaki Ammal, 1932 ; Martens and Vandendries, 1933 ; etc.)

Rosenberg (1909) also called the attention to the inconstancy of the chromocenters (prochromosomes). The inconstancy of the chromocenters was also noted by Tischler (1910) in studying an East-African *Musa sapientium*. He found that this plant has about as many chromocenters as the half of the chromosome number. Similar observations were reported by Suessenguth (1921) in *Dioscorea sinuata* and in *Thalia dealbata*. Tischler interpreted this phenomenon by assuming a secondary fusion of chromocenters from homologous chromosomes (cf. Tischler, 1934). The studies by Lundegårdh (1913, 1914) on *Vicia Faba* and *Allium*, by Sharp (1913) in *Vicia Faba*, and by de Smet (1914) in *Crepis* showed that the chromocenters concentrated into one side of the nucleus. The investigations by Vejdovsky (1926-27) and especially those by Heitz (1928 *a, b*, 1929, 1932 *a, b*, 1933 *a, b*, 1935) and Grégoire (1907, 1932) disclosed the causal behaviour of the chromocenters tracing the chromosomes from the previous cell division. Grégoire (1907) suggested first that the chromocenters represent those parts of the chromosomes which are lying near the centromeres. The appearance of the so-called "Kappenchromocentren" (Heitz) suggests the possibility of close association of more than two chromocenters, even from non-homologous chromosomes. Heitz and others showed that in some mosses sex chromosomes stain in the resting nuclei like the chromocenters. He assumed that they are inert, *i.e.*, their chromatin substance is "heterochromatic", while those that do not stain are active (euchromatic). In the somatic metaphase plates of highly distained hæmatoxyline preparations heterochromatin remains still deeply stained, while the euchromatin distains at first, thus the proximal regions (near centromeres) appear darker and the other parts—lighter. Heterochromatin cannot be distinguished by Feulgen reaction. It stains like the euchromatin. Various agents induced a more striking appearance of the heterochromatin in the resting nuclei. The chromocenters are especially strikingly pronounced in actively functioning cells which tend, so to say, to react, to the foreign inductors. *Drosera* tentacles represent one of the best examples in this respect, that were thoroughly investigated by Huie (1896, 1897, 1899) and by Rosenberg (1899, 1909). They found that in "resting stage" prophase-like chromatin figures are formed. Similar data were reported by Nicolosi-Roncati (1912) for *Pinguicula hirtiflora* and by Faber (1912) for *Nepenthes*. Similar phenomena were induced by Némec (1910) treating plant tissues by chemicals and

especially "diakinesis"-like nuclei were found by Winge (1927) in crown galls on *Beta vulgaris* which show clearly that abnormal conditions (in this particular case—the conditions created by the infection of beet tissues by *Bacterium tumefaciens*) may induce somatic "conjugations" and, I shall add, perhaps "crossing-over" in soma.

Significantly pronounced chromocenters have been observed in the cells of somatic tissues infected by *Mikorrhiza fungi* (Magnus, 1900. Shibata, 1902; Némec, 1910) and by *nodule bacteria* (Paratore, 1899, 1901; Wendel, 1917; Dangeard, 1926; Kostoff and Kendall, 1929; Kostoff, 1930; Hocquette, 1930, etc.) in *Papilionaceæ*. Faber (1912) found that in the cells of infected leaves of *Paveta* and *Psychotria* by bacteria "dicke chromosomenähnliche Gebilde auftreten, die sich stark mit Anilinfarbstoffen tingieren. Diese hyperchromatischen Kerne erinnern so an diejenigen die den Prophasen der heterotypen Teilung zu beobachten sind".

In plant galls, induced by various animal parasites, one can find, in the proximity of the parasites, cells with swollen nuclei having very pronounced chromocenters. I shall recall here the publications by Molliard (1897, 1900), Tischler (1901), Kostoff and Kendall (1929, 1930), Kostoff (1930 and, unpublished), Kendall (1930), etc. In these cases multinucleation is also accompanied with chromosome doubling and an increase of the number of chromocentres. Similar phenomena have been observed in the callus of heteroplastic grafts by Kostoff (1929–30) and in the endosperm produced following interspecific hybridization (Kostoff, 1930, unpublished). Jamaha (1927) observed such phenomena in treating *Vicia Faba* by various chemical agents and Kendall in treating *Pisum sativum*. Yamaha (1927) induced the same cell reaction in exposing *Vicia Faba* at 38° C. temperature. Somatic pairing of chromosomes and somatic chiasma formation were induced by Peto (1935) in *Hordeum* by high temperature. Chiasma formation in somatic cells was induced by Hearne (1936) in treating animal cells with cancerogenic agent methylcholantrene.

Special attention should be called to the nuclear and the chromosome reactions to X-ray treatments. Pekarek (1927) found that "das Erscheinen von chromocentrischen Gebilden...als wesentlichste Veränderung hervorzuheben ist", in X-raying *Vicia Faba*. Mavor and Svenson (1923, 1924) and Muller (1925) found that X-ray treatments increase the frequency of crossing-over in *Drosophila* near the centromere (spindle fibre attachment) and no change or even a decrease in the more distal parts. Cytological investigations by Heitz (1933, 1935), Painter and Stone (1935) Prokofieva (1936, 1937), Frolova (1935, 1936), etc., indicated that heterochromatic

segments are present at both sides of the centromeres. Correlating these cytological studies with earlier genetic and cytogenetic studies (Dobzhansky, 1929, 1930, 1932; Painter, 1931; Muller, 1932; Muller and Painter, 1932), upon the lack of fitness of the genetic chromosome map in *Drosophila* with the cytological one, which indicate, that the chromosomes have "inert" regions, *i.e.*, without genes, or with inert, inactive genes (*cf.* Muller, 1932; Heitz, 1935) some cytogeneticists called the heterochromatic regions as "inert" regions. (Some geneticists as Schultz, 1936, for example, called the term "inert"—a misnomer, considering the fact that inert chromosomes or chromosome segments have occasionally single genes, nevertheless this term is recently more often used in the cytogenetic literature than "heterochromatic".)

Heterochromatic regions stain darker than the other regions probably because in the heterochromatic regions the chromomeres that stain deeply dark are more closely situated and occasionally—somewhat larger. In studying recently the chromomeres in the somatic chromosomes of *Triticum monococcum* and especially of *Tr. Timopheevi* after fixing root tips in—platine chloride and commercial formalin fixation and staining in gentian violet I succeeded to differentiate very clearly the chromatids of the somatic chromosomes in chromomeres during the early metaphase (Fig. 1). There are some regions in the chromatids with much closely situated chromomeres while others have chromomeres situated at a much larger distance. Large chromomeres get further decomposed into smaller but closely situated. Hence one might conclude that heterochromatic regions stain deeply because the chromomeres in these regions are closely situated. In other words, the euchromatic regions have less chromomeres per length unit of the chromatid and longer interchromatic sections. The sections between each two chromomeres that do not stain with the gentian violet after platin chlorid—formol fixation will be called *genoneme*. This term was used by Koltzoff (1934) as a synonym for chromonema. Since the latter term is used broadly by cytologists and cytogeneticists and the term *genoneme* fits quite well to the easily distaining treads between the chromomeres, I think, it would be better to apply it for these sections. Koltzoff (1934) wrote: "It is difficult to decide what structure corresponds to the gene—the chromomere or the piece of *genoneme* between two chromomeres. The latter assumption seems to me more probable" (p. 313). The chromomere distribution in the heterochromatic (inert) regions in respect to the euchromatic ones gives sufficient background to advance the theory that the genes are located between the chromomeres (Kostoff, 1938).

It would be interesting to discuss further the behaviour of the heterochromatic regions. It should be mentioned first of all, that  $\gamma$ -rays of radium induce similar effects upon the chromosomes and upon their behaviour as Röntgen-rays.

In connection with the above data I shall recall here the experiments by Glass (1932) and Oliver (1932) which showed that the regions of the autosomes (Glass) nearer the spindle fiber and the right end of the X-chromosome (Oliver) of *Drosophila* are more likely to be "fragmented" by irradiation. These regions are the heterochromatic ones ("inert"). In the salivary glands the heterochromatic regions, *i.e.*, the chromocenters conjugate and form a "common chromocenter" (Painter and Stone, 1935; Prokofieva, 1935, 1937; Frolova, 1935, 1936, 1937, *etc.*).

Finally, the observations by numerous authors should be recalled which showed that X-ray treatment induce chiasma formation (bivalent chromosomes) in soma in the way high temperature does induce. I shall mention here the work by White (1935) who induced "bivalency" (Fig. 9 in White's paper) in *Locusta* by X-ray treatment during the mitosis which led to interchanges of chromosome segments that conditioned "fragmentations". Similar cytological figures and chromosome behaviour were attained by Riley (1936, Fig. II, 12) in X-raying *Tradescantia* and by Levan (1937) in X-raying *Allium*.

All the above given statements and a large number of the same kind, a part of which will be considered later, can be generalized in the following way: (a) The chromosomes of plants and animals have heterochromatic regions which stain deeply during the mitosis and even during the resting stage or prophase (chromocenters). (b) The chromatids during the early somatic metaphase, when properly fixed and stained can be differentiated into chromomeres the latter being much closely situated in the heterochromatic regions. (Regions with chromomere condensations have been found during the early prophase, which probably correspond to the heterochromatic regions.) (c) The heterochromatin is chiefly concentrated near the centromeres but some of the chromosomes have heterochromatic segments on the distal ends too as the observations in *Drosophila* (Prokofieva, 1935, 1937), *Crepis* (the author, 1938), *Triticum monococcum* (the author, 1938 in press) and other objects showed (Fig. 2). (d) External factors (chemical agents, parasites, X-rays, temperature, *etc.*) induce pronounced appearance of the chromocenters and a reduction in number, which is probably due to conjugation of the chromocenters. When an increase in number of the chromocenters has been observed, two interpretations can be advanced: (1) striking

appearance of the distal heterochromatic ends and (2) the same agents that have induced a pronounced appearance of the chromocenters, have also conditioned chromosome doubling. The occurrence of these two possibilities at the same time is not excluded. Cancerogenic agents (Hearne, 1936), parasites (Winge, 1927), X-rays (Marquardt, 1937), temperature (Peto, 1935), etc., induce chromosome association (often perhaps between non-homologous chromosomes) in somatic cells.

Somatic chromosome conjugation takes place in active glandular cells without any treatment (*i.e.*, in the salivary glands) where euchromatic homologous regions conjugate normally as they do during the meiosis, while the heterochromatic proximal regions of all chromosomes conjugate all together (non-homologous). The investigations by Prokofieva (1937) upon the structure of the chromocenter showed that the inert (heterochromatic) arm of the IV-chromosome conjugates with the heterochromatic regions of III- and II-chromosomes and the heterochromatic region of the X-chromosome (proximal) conjugates with those of IV- and II-chromosomes, *i.e.*, a conjugation of heterochromatic regions of non-homologous chromosomes. Genetic data obtained by Gershenson (in press) in studying the preferential segregation in triplo-IV flies (*Drosophila*) having XY + "fragmented" X also suggest a conjugation between non-homologous chromosomes probably in the heterochromatic (inert) regions. Kikkawa (1937) observations on the genetic behaviour of haplo-IV *Drosophila ananassæ* suggested a probable conjugation too between the heterochromatic regions of IV-chromosome and Y-chromosome. Meiotic chromosome conjugations, chiasma-formation and crossing-over, as a rule, result from an attraction between homologous parts carrying equal genes, *i.e.*, between like-parts (Jennings, 1923; Morgan, Bridges and Sturtevant, 1925; Muller, 1928; Dobzhansky, 1930, 1931, 1932; Dobzhansky and Sturtevant, 1931; Creighton and McClintock, 1931; Stern, 1931; Chino and Kikkawa, 1933; etc.). Synapsis that takes place between heterochromatic regions (also called inert regions) of the non-homologous chromosomes can be interpreted by assuming a likeness between these regions, no matter that they are distributed in all chromosomes. If we speak with genetic terms, they represent inactive "duplications" that can be found almost in each chromosome at the proximal ends and occasionally on the distal ones. Y-chromosome in *Drosophila melanogaster* is in its greater partheterochromatic (inert). It is genetically inert except of a few genes. Synapsis during the meiosis in normal diploids is chiefly regulated by the attraction of homologous active (euchromatic) parts. The hypothesis advanced by Snell (1938) for explaining

synapsis seems to us very plausible. In haploids with basic chromosome number as for example in *Triticum monococcum* ( $n = 7$ ) the chromosomes have not homologous members as they do have in polyploids or in secondary polyploids. In this haploid, however, end-to-end chromosome conjugation takes place as the works by Kihara and Katayama (1933) and by Chizaki (1933, 1934) showed. We studied the karyotype of *Tr. monococcum* for revealing the heterochromatic regions (unpublished) and found that the chromosomes have heterochromatic segments at the distal ends (Fig. 2). It is most probable then that the end-to-end chromosome conjugation in the haploid *Tr. monococcum* is due to a conjugation between heterochromatic segments of the distal regions. This case warns us to be more critical in drawing conclusions concerning the chromosome homology in species hybrids on the basis of partial, especially end-to-end chromosome conjugations. In reality, the terms "homologous" and "non-homologous" chromosomes appear in a new light. The contents of these conceptions evolve. It is now clear why the inert (heterochromatic) B-chromosome of *Zea* conjugates with itself by "folding back", or why three B-chromosomes conjugate in forming T-like figures as shown by McClintock (1933) excellent investigations. Randolph (1928) accumulated such B-chromosomes more than 25 in a plant without conditioning marked morphological effect. Avdulov (1937) found heterochromatic (inert) regions in the A-chromosomes (the active ones that have genes), which might also account for some "non-homologous" chromosome attractions and associations.

Biochemical and biophysical processes that proceed during the meiosis act in certain organisms somewhat differently on the chromosomes than those in the salivary glands since during the meiosis they usually induce synapsis between homologous chromosomes and between the heterochromatic regions of the homologous chromosomes, while in the salivary glands they induce close synapsis between homologous active parts and act upon the inert regions of all chromosomes in such a way that the inert regions conjugate altogether forming usually a common "chromocenter".

Association of heterochromatic regions in many haploids and species hybrids with asynapsis during the meiosis does not seem to be a rule, considering the fact that most of the haploids (*cf.* Kostoff, 1938) and many  $F_1$  species hybrids have asynapsis during the meiosis. On the other hand associations between "non-homologous" parts of A-chromosomes, and especially of B-chromosomes in *Zea*, association of the chromocenters of bivalents in *Vicia* (Enin, unpublished) forming sometimes a star-like figure (this occurs rarely), and end-to-end conjugations in *Triticum monococcum* haploids remind of the conditions in the salivary glands.

I wish to call the attention here to some observations made recently which will be of significance for our further discussions. In the  $F_1$  species hybrids *Nicotiana bonariensis* ( $n = 9$ )  $\times$  *N. Sanderæ* ( $n = 9$ ) I have found occasionally during the I and II meiotic anaphases one and rarely two chromatin bridges which undoubtedly result from crossing-over between inverted segments. Bivalents that appeared during the I meiosis of these species hybrids had quite often more than one chiasma per bivalent, which accounts for the formation of chromatids with two centromeres and a fragment without centromere during the II anaphase. The tapetum cells (the layer of somatic cells that envelopes internally the pollen sacs in which the pollen-mother cells develop) represent the cell barrier between the soma and the sac inside of which meiosis proceeds, *i.e.*, the place where processes proceed that induce chromosome pairing (*cf.* Kostoff, 1930). Tapetum cells have very active metabolic and catabolic processes. They expand enormously, their nuclei often divide once or several times, while the cells divide rarely; therefore they usually have more than one nuclei or polyploid nuclei: tetraploid, octaploid or even of higher polyploid grades. Normally, no chromosome conjugations take place in these cells. But sometimes conjugations occur. It is occasionally followed by interchange of chromosome segments. I have observed it rarely in pure species, but got the impression that it occurs more frequently in species hybrids. It occasionally occurred in the hybrid *N. bonariensis*  $\times$  *N. Sanderæ* as a result of which somatic anaphases with chromatin bridges (*i.e.*, chromatids with two centromeres) have been found (Fig. 3). In this case it is difficult to decide whether the

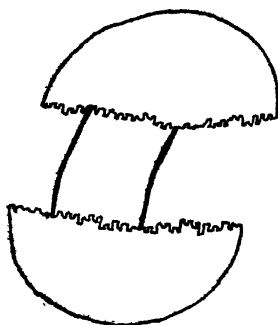


FIG. 3.

chromatids with two centromeres originate following somatic crossing-over between *bonariensis* and *Sanderæ* chromosome pairs which in meiosis behave as chromosomes having inversions, or interchange has occurred between heterochromatic regions of non-homologous chromosomes located in such places of the chromosomes, that chromatids with two centromeres get formed

after the interchange. A hybrid *N. Langsdorffii* ( $n = 9$ )  $\times$  *N. Sanderæ* ( $n = 9$ ), however, which I studied in 1931, offers a more definite answer. This hybrid formed normally 9 bivalents during the meiosis, while in the tapetum cells I have occasionally found anaphases with bicentric chromatids which can be interpreted by assuming interchange between non-homologous chromosomes.

The exceedingly small size of the fragment formed from interchange (I could not find it at all in most of the cases) in the tapetum cells of *bonariensis*  $\times$  *Sanderæ* can serve as an argument that crossing-over has taken place in the very distal ends of the chromosomes. The size of the fragments originating from crossing-over during the meiosis between inverted chromosome segments were in most of the cases much larger than those in tapetum. This suggests a possibility of interchanges in tapetum cells between extreme distal ends of the participating chromosomes. Some of the chromosomes have heterochromatic segments at the distal ends. Consequently, one cannot exclude the possibility of interchange between heterochromatic distal ends.

Some of the extensive data reported in the excellent paper by Stern (1936) upon the somatic crossing-over suggest strongly that in soma crossing-over occurs following conjugations between heterochromatic (inert) regions.

Stern (1936) wrote "the relative frequencies of somatic cross-overs in different regions of X-chromosomes are different from those of germinal cross-overs. Somatic crossing-over is more frequent near the fibre point. The presence of Minute— $n$  accentuates this shift" (p. 727). X-chromosome has a large inert region near the centromere. It does not seem improbable to suggest that the inert region is involved in these interchanges. This suggestion is also supported by the following statement made by Stern: "The X-chromosome duplication— $\theta$ —frequently undergoes somatic crossing-over with the X-chromosome—more frequently in the homologous right than in the homologous left regions. Germinal crossing-over involving  $\theta$  is very rare."

Gene Minute seems to act in a specific way creating conditions that induce chromosome synapsis in soma and crossing-over in the (or in the proximity of the) inert regions. According to Stern, "Somatic autosomal crossing-over takes place in both sexes, though more frequently in females. A peculiar specificness of the Minute effect leads to cross-overs in that arm of the third chromosome in which the Minute itself is located. Most cross-overs are concentrated near the fibre point region." The fibre point regions

are heterochromatic (inert). The effect of Minute gene is not great, therefore it is local. But genes with such a kind of effect (although somewhat greater) inducing crossing-over in heterochromatic chromosome regions, especially when it might also proceed between heterochromatic segments of non-homologous chromosomes might be decisive for cancer formation in case cancer results from "crossing-over" (interchange) between non-homologous chromosomes, as it seems to be so. (This problem I shall consider later again.)

According to Oliver (1934) "Somatic pairing is never normal, but is characteristic for the attraction of like parts" (*cf.* Dobzhansky and Sturtevant, 1931; Oliver and Van Atta, 1932; Sturtevant and Dobzhansky, 1930; Van Atta, 1932). The data obtained by McClintock (1933) and Jones (1936) suggest, however, crossing-over between non-homologous chromosomes in maize. In studying somatic segregation in maize Jones (1936) concluded: "The loss of a series of linked genes could be due either to non-disjunction, somatic crossing-over of homologous chromosomes, reciprocal translocations (non-homologous crossing-over), translocation or delation" (p. 166). If we correlate Jones' data with McClintock (1933) observations upon the conjugation of non-homologous chromosomes, which we interpreted above as conjugations between heterochromatin it is quite logical to interpret Jones' data by the assumption of interchange between heterochromatic regions of the non-homologous chromosomes.

Another case of such a somatic segregation seems to be the occurrence of white and red stripes on the pink corolla of the species hybrid *Nicotiana tabacum* (white corolla)  $\times$  *N. Sanderæ* (red corolla). Most frequently single white (1) or red (2) stripes have been found on the background of pink corolla but in a few cases I found a red and a white stripe (3) laying together on the background of pink. This somatic segregation can be also alternatively interpreted by interchange or by somatic non-disjunction but since they occurred too often and since studying the chromosome number in the pollen-mother cells (in about 250 cells of 10 floral buds) no cell with non-disjunction was found, it seemed more probable that somatic interchange between non-homologous (or partially homologous) chromosomes accounts for these somatic segregations (Fig. 4). If gene R is responsible for the red colour, being located in chromosome A, *i.e.*, AR, after a chromatid crossing-over between chromosome AR and BO ( $AR + BO = \text{pink}$ ),  $AR + AR = \text{red}$ ,  $BO + BO = \text{white}$ ), new chromosomes AO and BR should be formed. If cell with  $AR + BR$  chromosomes is capable to divide, red stripes should be formed; if cell with  $AO + BO$  is capable to divide further and reproduces

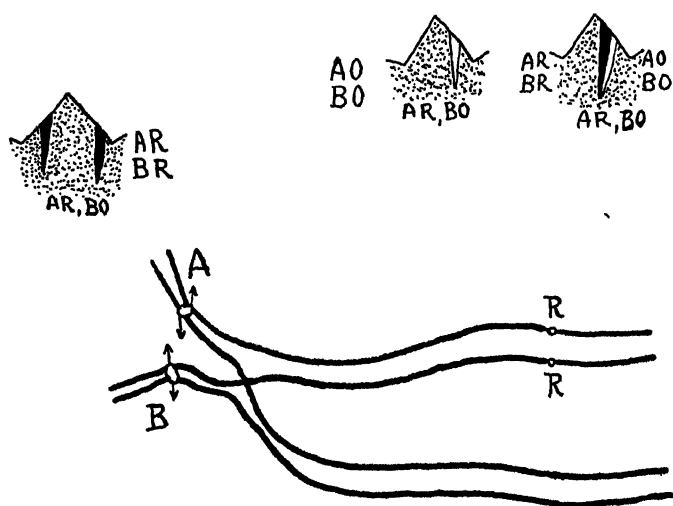


FIG. 4.

tissue—white stripes should be formed and, finally when both these kinds of cells divide, white and red stripes (adjacent) on the background of pink should originate. All these three kinds of stripes were observed. The variegations in the above-mentioned *Nicotiana* hybrids can be better interpreted by assuming interchange than non-disjunction for the following reasons. Cells with an interchange that do not lead to formation of acentric and bicentric chromatids have whole genomes of *N. tabacum* and *N. Sanderae* while those with non-disjunctions should have one whole chromosome in excess or one chromosome lacking; consequently the interchange cells should be viable and should be able to divide further and compete with the normal hybrid cells, while there is a very small probability that cells with “non-disjunctions” especially those that lack a chromosome would be able to compete with the normal cells. The formation of white and red areas suggest that the tempo of the cell division in these regions is like that in the pink normal regions.

The cytological investigations by Teleżiński (1935) on “unstable race” of *Petunia violacea* “with mosaic flower patterns”, showed that all of the plants of this race are structural heterozygotes (p. 233). He interpreted the “unstable” flower patterns by the hypothesis of “unstable genes”. It seems to me that his data can be much better interpreted by somatic interchanges.

It is quite possible that the great majority of the cases described as variegations as due to unstable genes represent chromosome interchanges in soma and that this interchange probably proceeds in the heterochromatic

regions. It is very desirable, of course, an arrangement of special experiments for verification of these suggestions.

In *Drosophila*, for example, Kaufmann (1934) observed configurations between the chromosomes in somatic cells that remind of chiasma formations in meiosis before genetic data were available upon the somatic crossing-over. The attention should be called here to the observations in *Drosophila* reported by Stern (1936) on the basis of which he stated that "Somatic crossing-over between X-chromosomes heterozygous for *bb* of inversion occurs within the inversion. It leads to a chromatid which possesses no fibre point and is thus eliminated, and to a complementary chromatid with two fibre points. This chromatid becomes fragmented and each fragment is included in a daughter nucleus" (pp. 727-28). It would be of great importance if such phenomena could be traced back to the exact point of crossing-over, *i.e.*, whether the somatic crossing-over has taken place in the active portion or in a small inert region. One could make a more general statement if in the excellent works by Schultz (1936) upon the variegation in *Drosophila* in relation to the inert chromosome regions and the increased numbers of Y-chromosomes, as well as in those by Stern (1936, 1936 *a*) and Neuhaus (1936) upon the crossing-over between X- and Y-chromosomes in *Drosophila*, and in that by Sturtevant (1937) upon the preferential segregation in triplo-IV females of *Drosophila melanogaster* such points were stressed. It is clear now that the majority of the data are in agreement with our postulates (some of them will be mentioned later), while some might not be for various reasons.

Somatic crossing over, when it occurs in species hybrids with asyndesis, is of a great significance from an evolutionary as well as from a practical point of view. *Secale* species as well as *Triticum* species have heterochromatic regions (Kostoff, Dogadkina and Tihanova, 1935; Kostoff, unpublished). The investigations by Lebedeff (1932), Müntzing (1935), Wakar (in press) and Kostoff (unpublished) upon the proceeding of the meiotic processes in *Triticum*  $\times$  *Secale* hybrids, especially upon the hybrids *Triticum vulgare* ( $n = 21$ )  $\times$  *Secale cereale* ( $n = 7$ ) showed that in some of these hybrids as a rule only 28 univalents appear, while in others (depending on the variety used from *Tr. vulgare*) one, two, three and sometimes even more bivalents are formed. This phenomena was explained before, that in the hybrids with bivalents an autosyndesis takes place during the meiosis between the chromosomes most probably from B and C (or as the Japanese school calls it D) genomes. In the light of the present studies it seems possible that in the hybrids with bivalents a somatic crossing-over between non-homologous

chromosomes has taken place, thus new chromosomes are formed, which have homologous segments. Such chromosomes can conjugate and form chiasmata. It is probable that the differences in the rate of somatic crossing-over that might be regulated by gene or genes like the Miniature in *Drosophila* (Stern, 1936) account for the differences in the behaviour of these species. It is also probable that somatic crossing-over should occur in heterochromatic regions or near them as it does in *Drosophila*. The chromosome conjugation during the meiosis as a result of somatic crossing-over can be easily explained in the following way: If *Secale* chromosome A with segments  $a, h, b, c, d$  conjugates with *Triticum* chromosome N with  $n, h, m, p, q$ ...segments, segment  $h$  being a heterochromatic one, and if interchange takes place in chromatid stage (as it seems to be so) the following four chromosomes should be formed  $a h b c d, a h m p q, n h b c d$  and  $n h m p q$ . Two of these chromosomes should go into one daughter cell and the other two into the other daughter cell namely:  $a h b c d$  and  $n h b c d$  to one pole and the other two to the other pole or  $a h b c d$  and  $n h m p q$  to the one pole and  $a h m p q$  and  $n h b c d$  to the other. If  $a h b c d$  goes together with  $n h b c d$  the segments  $h b c d$  are homologous and should conjugate during the meiosis. The same behaviour should have the chromosomes of the other daughter cell. Such plants, no doubt, should be chimeras. Another alternative interpretation should be that different varieties of *Triticum vulgare* have unequal heterochromatic regions in the karyotype, those with more, or with larger heterochromatic regions should form more bivalents. Species hybrids with asyndetic meiosis cannot be used for transferring of the characters from a species on the background of another one unless interchange (somatic or gametic) between non-homologous chromosomes takes place. This can be expected to occur most probably between the heterochromatic segments. Its frequency can be increased by external factors that increase the frequency of interchange (X-rays, radium, temperature, etc.), or in using varieties that have genes which act in a similar way gene Miniature acts in *Drosophila* as shown by Stern (1936).

The most effective agents which we know that increase the frequency of somatic chromosome interchanges seem to be X-rays (radium acts similarly) and temperature. The effects of the X-rays upon the chromosome interchanges is more thoroughly studied, therefore I shall consider here these data more extensively.

It is generally accepted now by a large number of the cytogeneticists that X-rays induce chromosome "breakage" and that the "broken" ends joint. Such is thought to be the mechanism of chromosome interchanges.

The cytological observations at various intervals of time after X-raying plants or animals show consistently that chromosome fragments are formed as a result of X-raying. It seems to me, however, that a large number of the fragments are results of chromosome interchanges (between homologous or non-homologous) and that the majority of the chromosome dislocations result from chromosome interchange between homologous or non-homologous chromosomes, in a similar way a crossing-over proceeds during the meiosis between "homologous" chromosome parts. The principal question that arises here would be : how X-rays do act upon the chromosomes and their milieu biochemically and biophysically ?

Our knowledge at the present time does not suffice to answer definitely this question, but some observations made upon this problem would help greatly for advancing the most probable working hypothesis. The important statement by J. Clark (1937, *Science*, No. 2229), that X-rays induce denaturations in the proteins regardless of alcalinity or acidity of the solution and of the temperature of which the experiment is performed, is of great significance for our discussion. In respect to the biophysical changes Seckt (1901) and Williams (1925) found that short exposure of various plants to X-rays increases the rate of the cytoplasmic streaming and Brownian movement, while longer exposure retards them. Radium rays have the same effect (Zuelzer and Philipp, 1925 ; Williams, 1925). Certain intensity and duration of exposure to X-rays cause an increase in the cytoplasmic viscosity (Seckt, 1902 ; Williams, 1923). The chromosomes are protein bodies and would react to the X-rays as proteins do react. High temperature increases also the cytoplasmic viscosity (Heilbrunn, 1924, 1928) and at a higher and longer exposure the proteins coagulate. Both these agents induce chromosome associations in somatic cells. The studies carried out by Marquardt (1937) supply good evidence in this respect. He has induced chromosome conjugation in *Bellevia romana* during the pollen mitosis by X-ray irradiation as his figures 2 and 4(a) show. The pollen have haploid chromosome number, nevertheless X-ray treatment induces chromosome association in polyvalent groups, which means that the conjugations take place between non-homologous chromosomes. The drawings by Marquardt can be interpreted as conjugations between regions near the centromeres and distal ends ; where the heterochromatic regions are usually located. Stone (1933) and Mather and Stone (1933) in studying the effect of X-rays on *Crocus* and *Tulipa root tips*, found that cells which actively divide are unaffected by irradiation. Marquardt (1937) came to similar conclusions, stating "dass in der Mitose vorhandene Translocationen nur während des Ruhestadiums erfolgen" (p. 151). These observations, supporting

Belling's (1933) and Darlington's (1932-37) theory of the chromosome division (or reproduction) during the prophase, suggest that Belling's theory (1933) of crossing-over during meiotic prophase, might be as well applied for these interchanges with great probability. I shall consider here, however, another alternative interpretation, since it seems to me that all observations, recorded by various authors, cannot be quite harmoniously unified. In studying the time of chromosome "breakage" in *Drosophila* Patterson (1935) concluded that breakage is not delayed even for a single cell generation (p. 241). Lewitsky and Araratian (1931) are inclined to interpret their results from the X-irradiation experiments in assuming "a marked postaction" of the X-rays in form of a durable chemical change in the plasma or chromosomes. Patterson's statements upon this question are somewhat contradictory. He (1933) crossed X-rayed virgin *Drosophilas* with untreated males. In studying the mosaic flies he concluded that their male parts lose the paternal X with the same frequency as the maternal X. This leads to the suggestion that the effect of radiation on the elimination of the X-chromosome is in part indirect, probably operating through the cytoplasm" (p. 51). In a more recent publication, Patterson (1935) drew the following conclusion: "We have been unable to find any evidence in support of this view that the effect of the irradiation may be indirect by the way of the cytoplasm" (p. 241).

Mather (1934) X-rayed *Vicia Faba* and *Tradescantia* and found "fragmentations" in the divisions occurring soon after treatment. The "fragments" in *Vicia Faba* appeared at late metaphase and anaphase while in *Tradescantia* before the metaphase. These differences he ascribed to the differences of forces that condition terminalization of chiasmata, *Vicia* having a much smaller terminalization coefficient. Riley (1936) found fragments at metaphase and anaphase of the microspore division within one hour after irradiation of *Tradescantia* buds and concluded that they should be "broken off" during the metaphase or late prophase. Riley (1936) reported that clumping of the chromosomes, treated during the meiosis and examined at the meiotic metaphase, was the only physiological effect he was able to notice. In his figures (especially fig. 12) drawn from the first pollen metaphase, chromatid exchange is shown between non-homologous chromosomes. The chromosomes are differentiated in length, the proteins of various chromosomes and genomes have different isoelectric points and probably coagulate (undergo denaturation, J. Clark, 1937) at various periods of time depending on the intensity and on the duration of exposure. The discontinuation of

the chromosomes probably occurs at the place where the proteins of the chromonemata coagulate partially or completely. Identical chromomeres have the same kind of proteins and should undergo changes at the same time, while the proteins of similar chromomeres should coagulate at about the same time in homogenous milieu. In the active regions "homologous" chromomeres (*i.e.*, equal) should coagulate at about the same time, consequently in more active segments chromatid discontinuation should occur at about the same time. But this does not always seem to occur, probably, because the chromosome milieu is very heterogeneous and the intensity of action of X-rays is not equal at any point. Some chromatid parts might join again at the place of discontinuation or with some of the ends of the chromatids from other chromosomes that are in proximity. Thus the effect of X-rays on the chromosome dislocations seems to be in two ways: (1) They induce association between chromosomes, often an association in the heterochromatic regions of the homologous or non-homologous chromosomes and the latter exchange parts. (2) They induce chromatid discontinuation, denaturing some proteins of certain chromomeres or in between them. (This process is known in the literature under the term "breakage".) Chromatid fragments of the same chromosome might join again with each other or with some fragments of chromatids of other chromosomes. The latter might occur more readily when X-rays induce conjugation between non-homologous chromosomes (point 1). The drawings given by Riley (1936) and Marquardt (1937) seem to fit quite well to the above advanced interpretations. It seems plausible to assume that the chromosomes of the heterochromatic regions of all chromosomes have closely alike proteins and they get denaturated at about the same time under the activity of X-rays, consequently, the discontinuations and junctions anew between non-homologous chromatids in these regions are to be expected more frequently. In other words, chromosome interchanges following X-ray treatments should occur most frequently in the heterochromatic regions.

The observations which showed that X-ray irradiations induce conjugation between non-homologous chromosomes suggest at the same time that the same agent upsets the force that prevents a coalescence of the like or similar parts of the chromosomes in the somatic cells. If the material per unit length of the heterochromatic regions of non-homologous chromosomes is less heterogeneous than the material of the euchromatic regions of two closely situated chromosomes, as it should logically be so, the coalescence should then more readily occur in the heterochromatic regions.

Some arguments that support the above given postulates will be later mentioned. Now I shall point out to the theories that attempt to interpret the mechanism of crossing-over and chromosome interchanges. I shall mention here the theory advanced by Serebrovsky (1929) who postulated a hypothetical tendency for chromosomes to become attached and subsequently "break" apart at different points. On this hypothesis, X-rays should increase the tendency for the chromosomes to become attached, or as we said above "coalesced". When this theory was advanced no cytological evidence was known in favour of such an attachment. I mentioned above that such observations were recently reported. It might be added here, that there seems to be a preferential chromosome attachment in the heterochromatic regions. This interpretation can be assumed for the cases when an attachment first occurs and then a discontinuation ("breakage"), it cannot explain, however the cases when first discontinuation and then reunion takes place. Patterson, Stone and Suche (1934) believed, "that most translocations, though not necessarily all, occur by an attachment followed by—breakage—" (p. 368). Muller called the broken-ends "sticky", consequently they should reunite. Stadler (1932) pointed out that simple translocations and terminal inversions have never been shown to follow irradiation and advanced the hypothesis that true ends cannot rejoin having a specific non-fusibility. Catcheside (1935, 1936), on the other hand, explained the reciprocity of structural changes without assuming a specific non-fusibility of the ends. He inferred that the reciprocity of changes is due to the high unlikelihood of an end of one chromosome lying near the point of discontinuation of another (*cf.* Darlington, 1937). The procedure of chromosome interchanges seems to be similar in many respects to that of the crossing-over. There are two hypotheses that attempt to interpret the procedure of crossing-over namely Belling's hypothesis (1933) and Darlington's hypothesis (1937). Belling postulated formation of new attachments between the newly formed chromomeres during the early prophase when the chromonemata of two homologous chromosomes associate. There are two serious arguments against this hypothesis: (1) The formation of new chromomeres, *i.e.*, the reproduction of the chromonemata does not seem to proceed at that stage since the chromosomes consist of two chromatids during the anaphase (Marshack, 1937; Atwood, 1937; Gates, 1937; etc.) and (2) on the basis of such a hypothesis very often acentric and bicentric chromatids should be formed unless special probable forces are postulated that regulate the junctions between newly formed chromomeres and disjunctions between the old ones. Darlington (1935, 1937), on the other hand,

assumes "breakage" in two of the four chromatids under the force of torsions. "The two broken ends will twist round their unbroken sister chromatids, thus releasing the coiling of the two which determined the breakage".—"The broken chromatids will reunite when, in the course of uncoiling, one of their ends first meets another. This will always be the end of a chromatid of a partner chromosome. Crossing-over will have occurred, and when the lapse of attractions leads to separation, a chiasma will appear" (p. 550). Darlington's hypothesis considers definite forces that are probably involved during the early prophase in connection with the spiralization and uncoiling of the chromosomes and chromatids as well as the chromosome attraction and repulsion. The weak point of Darlington's hypothesis is the postulate of chromatid "breakage," "uncoiling" and then "reunion". It seems very doubtful that such a "mechanism" will work so perfectly when the "axes" and "wheels" are built up from "colloid material" like that of the chromosomes. If we assume first a "breakage", i.e., discontinuation and then twisting, it seems very doubtful that the "broken" chromatids should always reunite, and that the reunion should always occur between partner chromatids. If Darlington's hypothesis was correct we should find very often fragments during the late metaphase and anaphase resulting from breakage but failure of reunion as we often find in irradiated material. The first objection to Belling's hypothesis is also an objection to Darlington's hypothesis. The intimate procedure of crossing-over as well as the chromosome conjugations and interchange of parts can be more satisfactorily interpreted when special studies are undertaken in the light of researches in the colloid chemistry in connection with the coalescence and miscibility of liquids. It seems that such a kind of research would throw light on the nature of the preferential interchange in the heterochromatic regions too.

I shall point out here to a striking correlation rather than coincidence between the position of the heterochromatic regions and the place of interchange following X-ray irradiation and temperature treatments. This phenomenon seems to occur in nature too. Preparations of root tips of *Crepis capillaris* fixed in chrom-formol fixatives, stained in iron-haematoxylin, and somewhat overdained showed a differential staining, the proximal parts being darker and often small portions of the distal ones (Fig. 5). Similar differential stainings can be seen on the microphotographs reported by Matsuura (1937, his figs. 1 and 2). When one compares the chromosome interchanges in *Crepis capillaris* induced by X-ray irradiation and reported by Lewitsky, Shepeleva and Titova (1934) as well as the new karyotypes that

might be derived from such having A-, C- and D-chromosomes and compared with the karyotypes found in nature and reported by Babcock (1936), one gets the impression that the majority of the newly originating

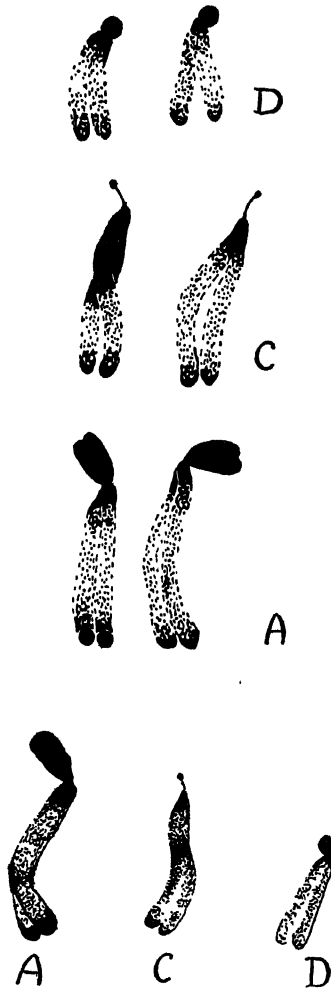


FIG. 5.

karyotypes resulted from chromosome interchanges in the heterochromatic regions (distal, proximal, or both) of non-homologous or homologous chromosomes, or finally—resulting from interchanges involving only one chromosome, *i.e.*, following “fold backs”. In Fig. 6 it is shown diagrammatically how an interchange might take place in the proximal regions involving one chromatid of A- and one of D-chromosomes. This way new chromosomes have obviously resulted, one with two large arms ( $a_1 d_1$ ) and another with

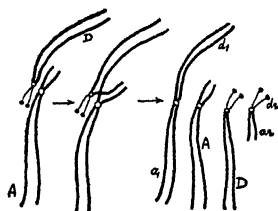


FIG. 6.

small arms ( $a_2$   $d_2$ —, Fig. 6) in Lewitsky's experiments (1934, his fig. 4). In the other diagrams (Figs. 7, 8, 9, 10) I have drawn only the chromosomes

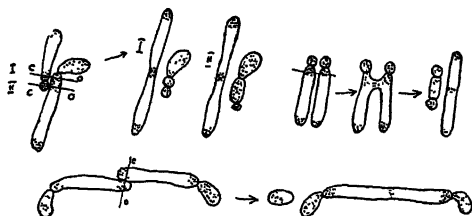


FIG. 7.

and the new types that would originate from interchange ( $c - o$ ) in the heterochromatic regions. When interchanges occur in the regions as shown in Fig. 7 acentric and bicentric chromatids and chromosomes will be formed, both being not adapted to survive, the former, because it has not a centromere that regulates its normal behaviour, the latter, because it has two centromeres one often pulling to the one pole while the other to the other pole. Cells with discontinued bicentric chromatids can sometimes survive and give rise to new individuals and even new karyotypes (Stern, 1936; Kostoff, unpublished). Sometimes whole chromosomes can originate consisting chiefly from heterochromatic material (Fig. 8,  $m$ ,  $m_1$ ). This suggests the

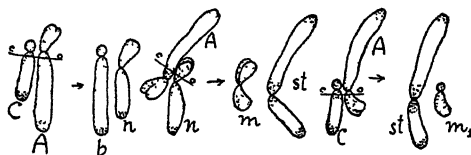


FIG. 8.

idea that Y-chromosome in *Drosophila melanogaster* probably consists chiefly of inert material of X-chromosome. The production of a five-chromosome race of *Drosophila melanogaster* by Schultz (Morgan, Bridges and Schultz, 1935) is in favour of this idea. From interchange in the proximal heterochromatic regions of A- and C-chromosomes in *Crepis* (Fig. 8) two

new chromosomes could be formed  $b$  and  $n$ . A chromosome pair like " $b$ " occurs in the species *Crepis bungei*, chromosome pair like " $n$ " can be found in *Crepis nicaensis* and *C. albida*. If an interchange takes place further in the heterochromatic regions as between " $n$ " and " $A$ "-chromosomes as shown in Fig. 8, two new chromosomes: " $m$ " and " $st$ " can be formed. One chromosome pair, similar to chromosome " $m$ ", was found by Babcock in the species *Crepis montana* and *C. senecioides*; chromosome pair, morphologically similar to chromosome " $st$ ", was found in *Crepis Stoyanovii*. An interchange between chromosome C and A (fig. 8), when the distal ends are turned to a reciprocal direction, chromosome can be formed like " $m_1$ ", that is found in *Crepis montana* and like " $st$ "-chromosome in *Crepis Stoyanovii*.

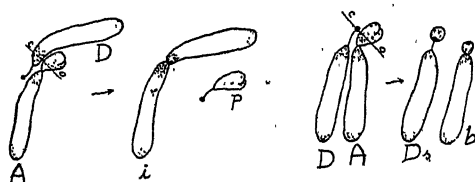


FIG. 9.

When interchange takes place in the proximal regions between A- and D-chromosomes two possibilities are present: (1) When the distal chromosome ends point to quite opposite directions (Figs. 6 and 9 left), chromosomes " $i$ " ( $a_1 d_1$ ) and " $p$ " ( $a_2 d_2$ ) can be formed, type " $i$ " being found in *Crepis incarnata*, *palestina*, *gymnopus* and *pulchra* and " $p$ " in *Crepis parviflora* (comp. Babcock, 1936). (2) When the distal ends of the chromosomes point to one and the same direction (Fig. 9, right) chromosomes " $D_s$ " and " $b$ " can be formed, " $D_s$ " reminding of a pair of *Crepis setosa*; " $b$ " of a pair of *Crepis bungei* and *incarnata* (cf. Babcock, 1936). The attempts made above to interpret the origin of some chromosomes is not an attempt to solve phylogenetic problems in the genus *Crepis*, since the reverse process is also possible, as for example, the formation of A- and B-chromosomes from " $i$ " ( $a_1 d_1$ ) and " $p$ " ( $a_2 d_2$ ) chromosomes, etc. "Folding backs" (Fig. 10) can also lead to chromosome reorganization. The above schemes as well as the results obtained by Lewitzky and his co-workers (1934) suggest that chromosomes with two arms are not necessarily more

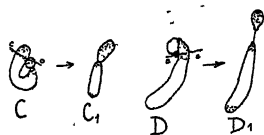


FIG. 10.

primitive, from a phylogenetic point of view, since they can originate at any time by interchanges.

Chromosome reorganizations, like those conditioned by X-ray irradiations were induced in *Crepis* by Shkvarnikov (1936) in applying abnormally high temperature. His fig. 2, in which some types of chromosome interchange in *Crepis capillaris* are given, suggests strongly preferential interchange in the heterochromatic regions.

Another factor that increase the interchange between the chromosomes is the aging. Heribert Nilsson (1931) noted that in *Oenothera*: "*Mit dem Alter des Samens, also mit der herabgesetzten Keimfähigkeit, geht das Ansteigen des Mutationsprozents ausgesprochen parallel*" (p. 326). *Oenothera* mutations are most frequently chromosome alterations. More recently Navashin (1933-36) and his co-workers have shown that with the aging of the seeds of *Crepis* and other plants the chromosomal "mutations" are significantly increased. The new type of chromosomes formed also remind of those formed under the activity of X-ray irradiations and those at high temperature. The formation of bicentric chromosomes following interchange most probably in the distal ends, as shown in our diagrams (Fig. 7) might account for the chromatin bridges observed by Navashin and Gerassimova (1935). Their drawings also suggest a preferential interchange between non-homologous chromosomes in the heterochromatic regions (N. and G., 1935, their figs. 18 and 19). Chromatin bridges were found by Barber (1938) during the second post meiotic mitosis in aged *Pæonia* and *Kniphofia* pollen. He supposed, however, that bridges originate by union of the homologous ends of the two sister chromatids derived by division from one parent chromosome. Barber's interpretation is possible, but it seems to me that the possibility of interchange between non-homologous chromosomes is not excluded.

It was pointed out that X-rays induce denaturation of the proteins (Clark). The aging seems to be accompanied with similar biophysical changes. The works by Ružicka (1922 and later) and his students showed that aging is accompanied with changes in the isoelectric points of the protein colloids which allows an easier denaturation (protoplasma-hysteresis).

In the light of the present discussion, the "mutations" of *Oenothera* that often occur can be better interpreted as interchanges in the heterochromatic regions of non-homologous chromosomes, aging being the factor that increase the rate of these interchanges.

In all cases of interchanges that are accompanied with protein denaturation it is possible that this phenomenon facilitates the coalescence of the chromatids and further interchanges when chromatid separation proceeds.

I shall finally mention of the interchanges that occur occasionally in centrifuged material. Centrifugal force transmits the chromosomes towards the centrifugal end of the cell and can mechanically facilitate a coalescence between some regions of non-homologous chromosomes.

The rôle of the heterochromatic regions can be evaluated in two ways from a phylogenetic point of view: (1) The heterochromatin might be the place where new genes originate. When they are totally inert a deficiency in these regions would not affect the vitality as well as the hereditary complex (*cf.* Kostoff, 1938a; Demerec and Hoover, 1936). By interchanges the heterochromatic regions can be transmitted from the proximal at the distal ends. (2) Interchanges in the heterochromatic regions lead to duplications of segments (active as well as inert). The evolutionary significance of the duplications was stressed recently by Morgan, Bridges and Schultz (1935). They supplied evidence which "confirms the hypothesis that the normal evolutionary increase in number of genes has been by 'duplication' of the previously existing genes, followed by diversifying mutation" (p. 287). Reduplications were found by many authors (Bridges, 1935; Kossikov, 1936; Offermann, 1936; Grünberg, 1937; etc.) and most of them share this opinion.

Closing the discussion I shall call once more the attention to the most probable origin of the atypical growth in plants and animals. The cancerogenic agents as methylcholontrene (Hearne, 1936) and *Bacterium tumefaciens* (Winge, 1927) induce chromosome figures that remind us of chromosome association during the prophase. Such a chromosome behaviour obviously leads to somatic crossing-over and interchange between non-homologous chromosomes. These phenomena were found in animals as well as in plants (Stern, 1936; Jones, 1937; etc.). I showed above that chromosome alterations, most probably interchanges in species hybrids, occur quite often. Species hybrids like *Nicotiana glauca*  $\times$  *Langsdorffii*, *N. paniculata*  $\times$  *Langsdorffii*, *N. rustica*  $\times$  *Cavanilliesii*, *N. glauca*  $\times$  *longiflora*, etc. form large tumors (Kostoff, 1935, 1937, etc.). The chromosome number in the tumors is usually equal to the somatic chromosome number and rarely chromosome duplications and aneuploidy has been found. Hence it seems most probable that atypical growth results from interchanges between non-homologous chromosomes and it probably occurs preferentially in the heterochromatic regions.

Discussing the data upon the heterochromatin and its behaviour recorded by various workers and adding some new observations into this line of work I attempted to correlate a series of phenomena connected with chromosome

interchanges and crossing-over. In doing this a number of the existing theories and hypotheses were criticised in the light of new researches. For a series of phenomena new interpretations were given. Some of them should be considered at the present time as working hypotheses, others having more solid experimental background can be treated as theories that generalize the knowledge at the present time in the respective fields of research.

Extreme empiricists do not often evaluate correctly the significance of the theories which mobilize the present knowledge in a certain field of the biological sciences and stimulate research into most promising directions.

As a controversion to the strict empiristic conceptions in experimental biology I shall quote Willstätter's opinion which seems to express much better the value of hypotheses in the experimental research work than a sceptical denial of any stimulating ideas which might lead to a disarmament, to a standstill. Willstätter wrote: "It is not important for the scientist whether his own theory proves the right one in the end. Our experiments are not carried out to decide whether we are right, not to prove that we are right, but to gain new knowledge. It is for knowledge's sake that we plow and sow. It is not inglorious at all to have erred in theories and hypotheses. Our hypotheses are intended for the present rather than the future. They are indispensable for us in the explanation of the secured facts to enliven and to mobilize them and above all, to blaze a trail into unknown regions towards new discoveries." But there is no doubt, that it is the experiment which finally decides; it proves or disproves the theory and hypothesis.

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## DESCRIPTION OF FIGURES.

- FIG. 1.—Microphotographs from two cells at several levels from the root tips of *Triticum timopheevi* during the mitosis. Roots fixed in fixation platine chlorid and commercial formalin fixation and stained in gentian violet. Five pictures at the right side are taken with lower magnification than the pictures at the left. *Note*: (1) Chromonemata consist of: (a) chromomeres that stain; and (b) substances in between that do not stain. (2) In some regions (usually distal and proximal) the chromomeres are darker and more closely situated.
- FIG. 2.—Several chromosomes drawn separately from one and the same metaphase plate from *Triticum monococcum* (It is aimed to show the lighter and the darker regions). *Note* that the distal ends stain darker.
- FIG. 3.—Late anaphase from a tapetum cell of the hybrid *Nicotiana bonariensis*  $\times$  *N. Sanderræ*. *Note* two chromatin bridges representing bicentric chromatids.
- FIG. 4.—Parts from the flowers (corollas) of the hybrid *Nicotiana tabacum*  $\times$  *N. Sanderræ*. *Note*: (1) Left—Black (red) stripes (AR, BR) on the punctured (pink) background (AR, BO); (2) In the middle—White stripe (AO, BO) on the punctured (pink) background (AR, BO); and (3) Right—Black (red) and white stripes on the punctured (pink) background. Down—diagram showing the probable origin of the stripes as a result of a chromatid crossing-over in soma (corollas) between A-chromosomes carrying the gene for red (R) and B-Chromosome (*cf.* text).
- FIG. 5.—Differential staining of the chromosomes in *Crepis capillaris* after Lewitzky's chrom-formol fixation and Hæmatoxylin staining.
- FIGS. 6, 7, 8, 9 and 10.—Diagrams showing the mode of origin of new chromosomes following chromatid interchanges between non-homologous chromosomes in the heterochromatic regions (*cf.* text).

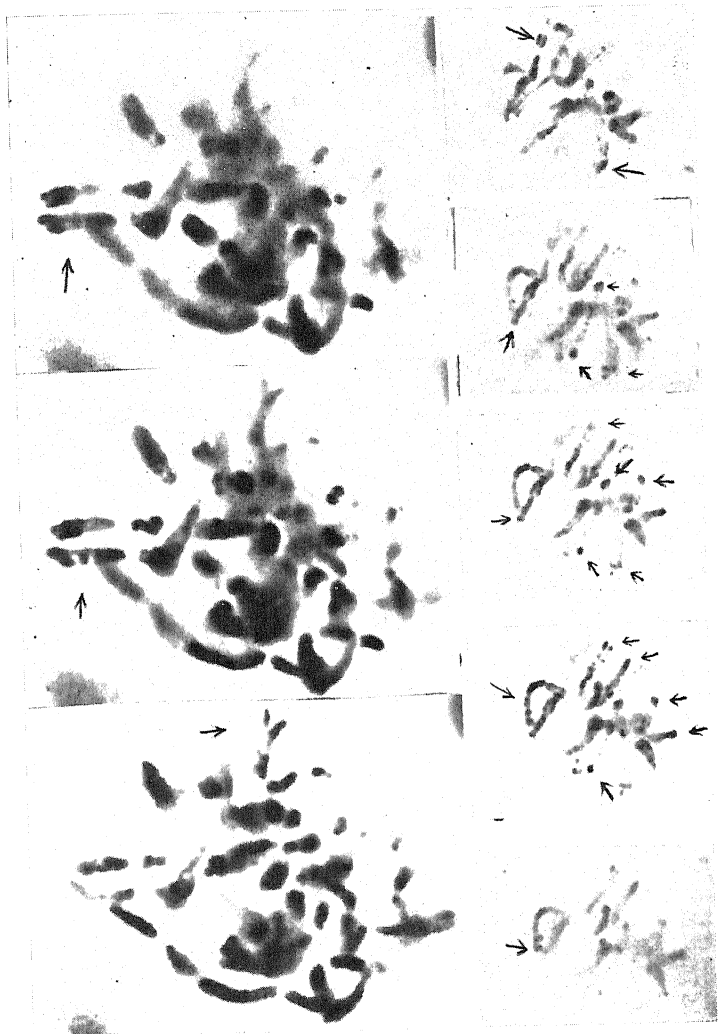


FIG. 1.



FIG. 2.



## STUDIES IN THE PROTEACEAE.

### II. Floral Anatomy and Morphology of *Macadamia ternifolia* F. Muell.

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#### *Introduction.*

It has already been pointed out in a previous paper on *Grevillea robusta* Cunn. (1938 b), in which a review of the important contributions has been given, that the literature pertaining to floral morphology in the members of the Proteaceæ is very meagre. The number of investigated genera is very small in proportion to the total number belonging to the family. The present contribution is intended as an addition to our knowledge of the family at least to some extent.

Not only on account of the systematic position of the family which is much disputed, but also, to a larger extent on account of the encouraging results obtained in *Grevillea robusta* Cunn. (Kausik, 1938 b), the present investigation was undertaken with the hope that many interesting facts of floral morphology remain yet to be recorded in the family. This hope has not been frustrated in the present case.

Locally, only three isolated plants of the species under examination here, *Macadamia ternifolia* F. Muell., the Queensland Nut Tree, are available in the Government Horticultural Gardens, Lal-Bagh, Bangalore. The material was collected from these plants and killed in Bouin's fluid. Sections were cut at different thicknesses to suit the requirements of study. Considerable difficulty was experienced in cutting on account of the presence of abundant tannin in the floral parts and the general hardness of the flowers even in early stages of development. In the case of the developing fruits there was less difficulty in sectioning, as then the material could be easily handled and the outer hard portions of the ovary removed. Heidenhain's iron-alum Hæmatoxylin was used throughout, but in the study of floral anatomy, alcoholic saffranin and a counterstain, for contrast, of light green dissolved in clove oil were employed.

*The Structure of the Flower.*

The flowers are borne in pairs on the main axis of the inflorescence, which is a raceme. Each flower has a long pedicel and consists of four perianth lobes, opposite to which are four stamens. The filaments are adnate to the perianth lobes throughout their basal portions and are free only a little below the anthers. The perianth lobes meet each other along their margins by dove-tailing arrangements (Figs. 14, 15). Slight constrictions are found between the bases of the perianth lobes and the receptacle of the flower (Fig. 14) where the two separate at the time of the opening of the flowers. Within the perianth is a large nectar-secreting disc (Figs. 3, 4 and 14) in the form of a collar at the base of the ovary.

The ovary, which is made up of a single carpel and contains two ovules, is raised on a short gynophore-like stalk (Fig. 14) and ends distally in a long slender style terminated by a large stigma. The wall of the ovary is densely clothed over with short thick-walled hairs, which have basal joint cells (Figs. 20, 21). The hairs break away at the joints as the fruits begin to develop.

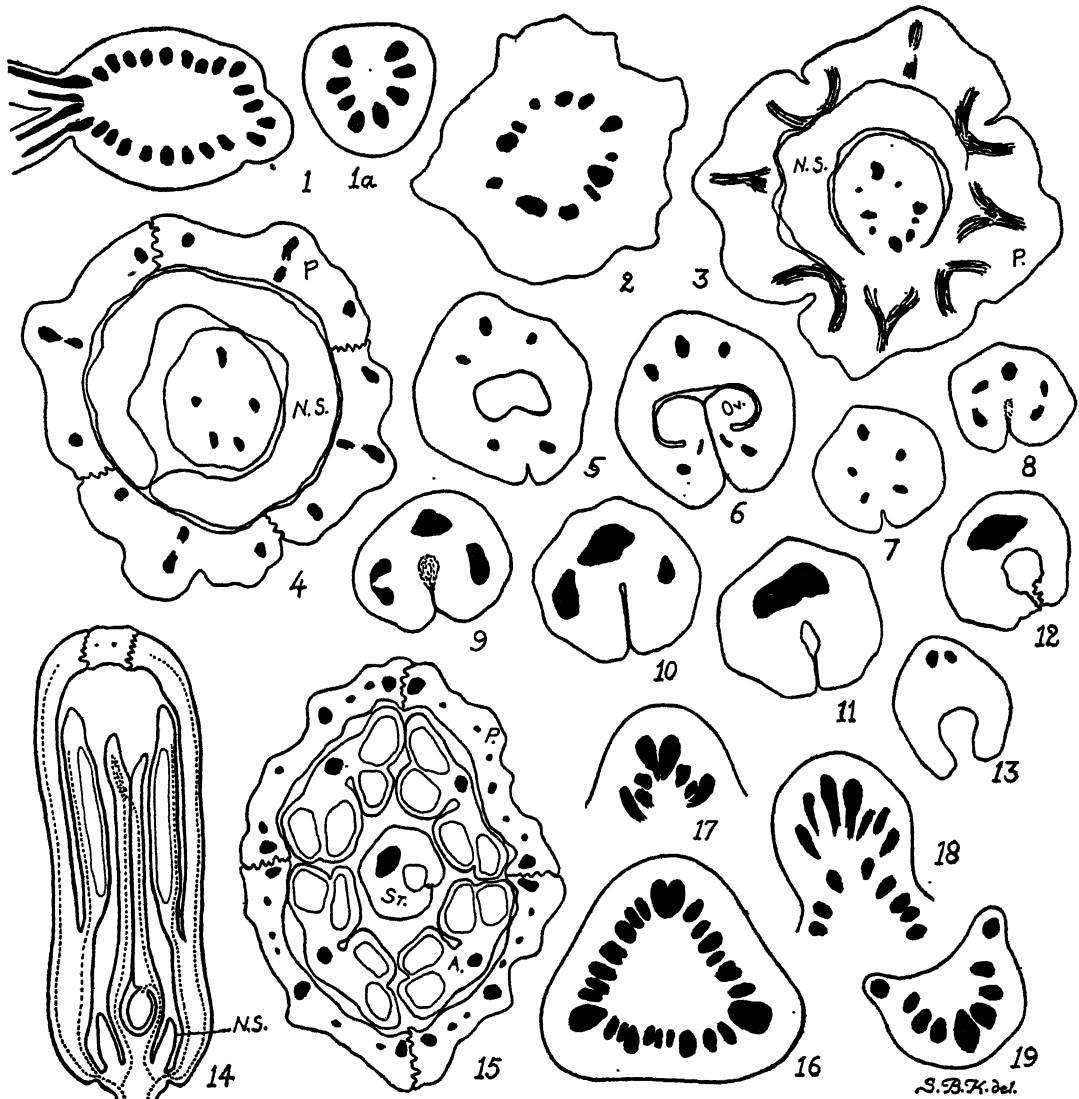
In the formation of the ovary, the margins of the carpel are folded inwards on the ventral side, where a longitudinal groove is present. This groove suggests that the fusion of the margins is not quite complete. The groove continues into the style and the stigma. In the latter a small pore-like opening is present along the groove (Fig. 23) and beyond this, the stigma opens out like a funnel, the inside of which is covered over by numerous papillate cells for conducting the pollen tubes (Fig. 22). In a longitudinal section of the ovary, each margin of the inrolled carpel shows a small overhanging ridge of cells above the attachment of the ovule (Figs. 14, 20).

There are large groups of tannin-filled cells in all the floral parts, including the outer integument and the chalazal region of the ovule.

*Vascular Anatomy of the Flower.*

The vascular tissue of the flower separates out from the vascular cylinder of the main axis of the inflorescence in the form of a dorsiventral arc consisting of a few bundles (Fig. 1). The arc gradually closes up to become radial (Fig. 1 a) and forms a ring of vascular strands as soon as the pedicel becomes distinct from the main axis (Fig. 2). The vascular ring of the pedicel continues into the receptacle of the flower, where it gives rise to the traces for the several floral organs.

The first set of traces to be separated from the receptacular stele is that for the perianth lobes. In this, there arise four paired and as many single strands, the former alternating with the latter (Fig. 3). The strands of the respective pairs fuse with each other and enter the four perianth lobes



FIGS. 1-19.—Showing vascular supplies to the floral organs; see text for a detailed description. Fig. 1. Separation of the floral vascular tissue from the cylinder of the main axis; Fig. 1a. The same at a higher level. Fig. 2. Vascular ring in the pedicel. Figs. 3-13. The vascular traces at successively higher levels in the flower. In Figs. 5-13 the outer floral parts are excluded. Fig. 14. Longitudinal section of flower showing course of vascular strands. Fig. 15. Transverse section of same to show general arrangement of parts and their traces. Figs. 16-19. Showing separation of foliar trace for comparison.

as four large median strands. Each of the alternating single strands forks into two limbs as it passes out to the periphery of the receptacle and the two limbs enter the margins of two adjacent perianth lobes. Thus each

perianth lobe has essentially three main strands at its base, the median of which is a double strand, while the two marginals are half strands, those of adjacent margins of two perianth lobes being derived from a single strand in the receptacle. These three main strands branch higher up as they traverse the perianth lobes (Fig. 15).

The vascular supplies of the androecium consist of four large strands, one for each stamen and derived from the large median strand of the corresponding perianth lobe on the ventral or adaxial side (Fig. 4). The stamen traces are separated at the very base of the perianth lobes and enter the inner median ridges of the latter, which are formed by the fusion of the filaments and the perianth lobes. The stamen strands are throughout single and become enclosed in a sheath of tannin cells as they enter the anthers (Fig. 27).

The nectar disc does not receive any vascular traces from the receptacle. It is therefore regarded that it has no morphological relationship with the other floral parts. Such a conclusion is also reached by Brough (1933) in *Grevillea robusta* Cunn.

After the departure of the perianth traces, a ring of vascular tissue, consisting of five large and a few smaller strands is left over in the stele (Fig. 3). The smaller strands ascend only a short distance into the stalk of the ovary and become exhausted (Fig. 4). The five larger ones, on the other hand, pursue their courses beyond and enter the single carpel of the ovary where they assume definite positions as a large median dorsal, two median lateral and two ventral or marginal strands (Fig. 5). The ventral strands after giving rise to the ovule traces (Fig. 6), get into the style along with the dorsal and the median lateral strands (Fig. 7). The courses of these strands remain unaltered throughout the entire length of the style, but at the region of the stigma, the ventrals shift themselves towards the median laterals with which they fuse (Figs. 8-10). Finally the fused strands again recede towards the dorsal side of the stigma and unite with the dorsal strand to form a large stigmatic vascular mass (Figs. 11, 12). The latter ultimately disappears at the tip of the stigma, but immediately prior to this shows a slight forking into two short stumps (Fig. 13).

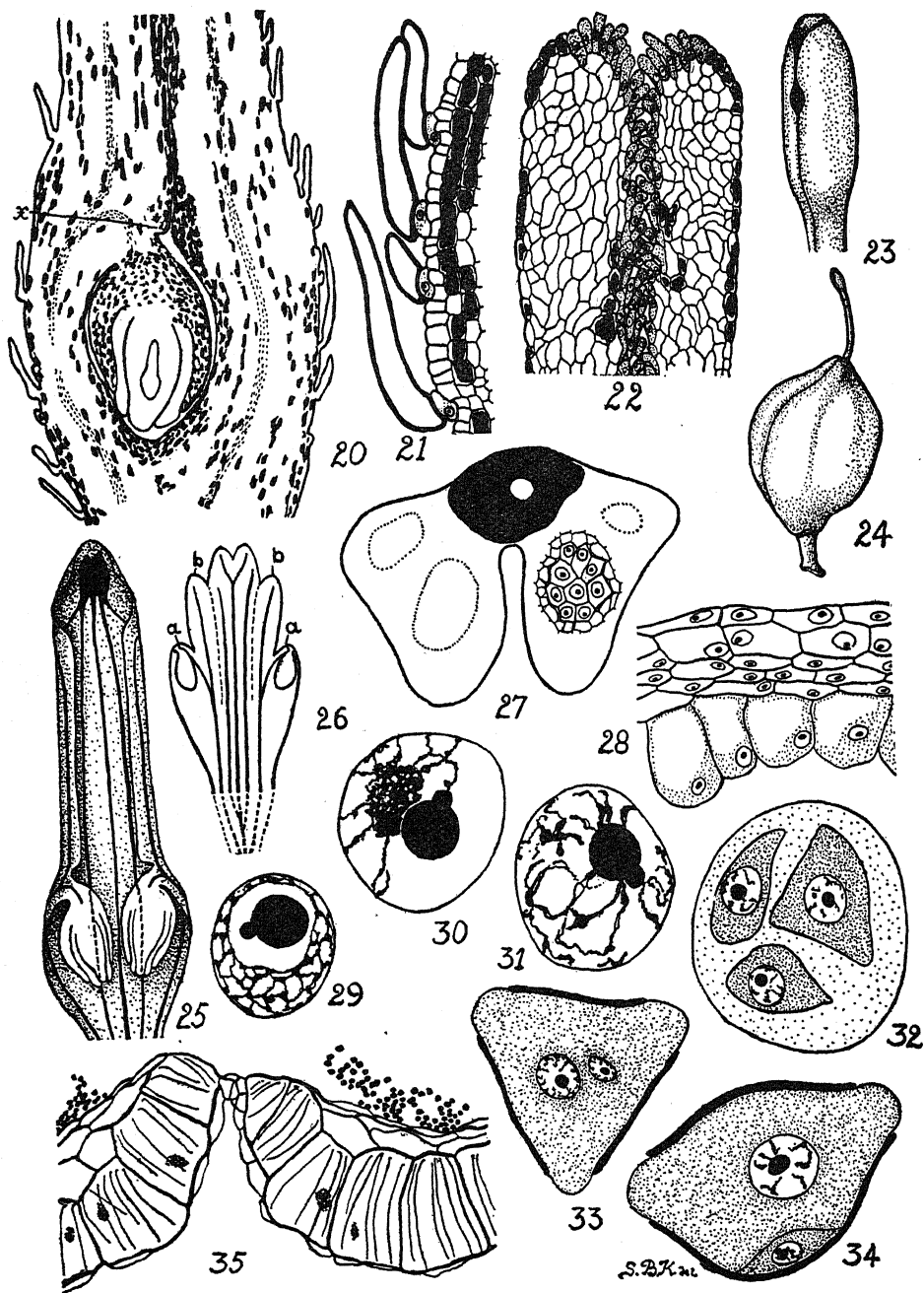
It may be worthwhile at this stage to point out certain significant features in floral structure. The separation of the floral vascular tissue in the form of a dorsiventral arc is so similar to the separation of a leaf trace (Figs. 16 to 19) or a branch trace in the vegetative portions that perhaps rigid distinctions cannot after all be placed between the axial and appendicular organs of the shoot. One may therefore conclude with Arber (1930)

that fundamentally there are only two morphological entities, the shoot and the root.

In the separation of the large median vascular strands of the perianth a similarity seems to exist with the leaves, where too a double median strand (Figs. 16 and 17) is conspicuous. Eames (1931) regards that the vascular supplies of the sepals are similar to those of the foliage leaves of the plant. In agreement with this, the perianth lobes are regarded as sepals. The alternating single strands supplying the margins of adjacent perianth lobes are perhaps really petal traces, the courses of which have become altered on account of a complete disappearance of the whorl of petals. These traces therefore enter the lobes of the only surviving floral envelope.

Lastly, the nature and construction of the ovary in *Macadamia* F. Muell. are such that they may be considered in the light of the interpretations of Hunt (1937) regarding the origin of the modern Angiospermic carpel. Hunt visualises the forerunner of the carpel as an essentially three-lobed structure, the lobes dichotomising and possessing independent vascular branches. In the carpel of *Macadamia* F. Muell. indications are seen of such a carpel with three lobes (Fig. 25), the two laterals of which alone show a forking into two branches, while the central is undivided. The forking of the stigmatic vascular mass into two short stumps is perhaps the only evidence of the original branching tendency of the central lobe that is persisting. The condition of the Protead carpel, at least in *Macadamia* F. Muell., might have resembled at some time the condition of the primitive carpel shown in Fig. 26. Here the outer branches of the lateral lobes (marked *a*) have become specialised to ovule-bearing and so have closed over gradually, while the inner of the same (marked *b*) have fused all through their length with the margins of the central lobe to form the style and the stigma. The overhanging ridge of cells above the attachment of the ovules (as seen in a longitudinal section) and the pore on the stigma formed on account of notches in the apposing margins of the carpel are perhaps the only lingering remnants of the regions separating the branches and the lobes of the primitive carpel.

The vascular structure of the ovary supports the above interpretations of the carpel to a large extent. The dorsal strand is not only more strongly developed than either of the other two pairs, but also takes part in a larger measure in the formation of the stigmatic vascular tissue, towards which the other strands converge. The forking of the stigmatic vascular tissue suggests the original branching tendency of the central lobe, which remains externally simple. The ventral strands in the style belong to the inner branches of the lateral lobes which have participated in the formation of the



FIGS. 20-35.—Fig. 20. Longitudinal section of ovary showing the atropous pendulous ovule and the hairs on the ovary wall. Note the overhanging ridge of cells above the ovule attachment marked *x*. The black areas represent tannin cells. Fig. 21. The hairs on

the ovary wall enlarged to show the joint cells.  $\times 56$ . Fig. 22. Longitudinal section through stigma showing glandular conducting cells.  $\times 120$ . Fig. 23. Stigma enlarged to show pore along the ventral groove. Fig. 24. Fruit showing the two elevations on either side and in front of the style. The ventral groove is also seen. Fig. 25. Ovary shown as if opened out and laid flat. Fig. 26. Diagrammatic construction of carpel. Note the similarity between this and the ovary shown in Fig. 25. Fig. 27. Transverse section of anther. A sheath of tannin cells surrounds the vascular strand.  $\times 160$ . Fig. 28. Part of wall of young anther.  $\times 900$ . Figs. 29–31. Some of the prophase nuclei of the microspore mother cells, showing bud of nucleolus.  $\times 2700$ . Fig. 32. Three of the four microspores within the mother cell wall.  $\times 1800$ . Figs. 33 & 34. Pollen grains. Fig. 33.  $\times 1350$ , Fig. 34.  $\times 1800$ . Fig. 35. Part of wall of mature anther.  $\times 900$ .

style, while the ovule traces are the vascular strands of the extremely consolidated outer branches, which alone bear the two ovules.

In the developing fruits two small elevations are found (Fig. 24) on either side and in front of the style. These elevations are regarded as representing the inflated distal portions of the outer branches which became inrolled to form the ovary.

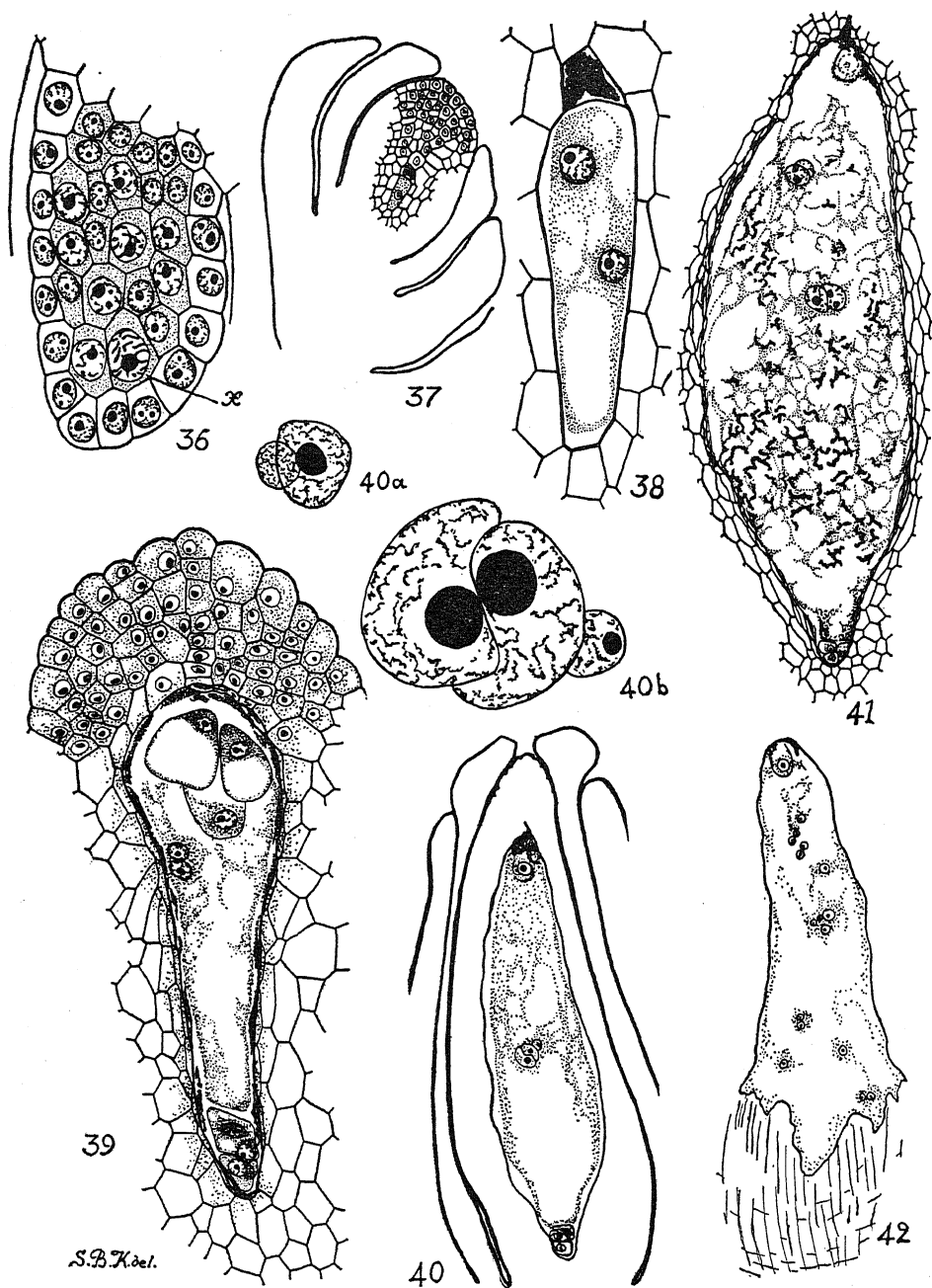
#### *The Structure of the Anther and the Formation of the Microspores.*

The anther is extremely elongated and its wall shows as usual, the epidermis, endothecium, three middle layers and the tapetum (Fig. 28). The tapetal cells though large, are not very conspicuous even in the earlier stages, as for instance when the prophase changes are taking place in the microspore mother cells. Each tapetal cell has a large vacuole, a small quantity of cytoplasm and a single nucleus, which does not divide further.

The meiotic divisions in the mother cell nuclei in the formation of the microspores are normal. It may be noted here that the nuclei possess a single large nucleolus, with which is always characteristically associated a bud (Figs. 29 to 31). In most of the nuclei of the mother cells of the young anthers synizesis, whether regarded as an artifact or not, seems to be a very constant feature of considerable duration.

With the formation of the microspores, the original mother cell wall swells up and becomes gelatinous (Fig. 32) before completely disappearing and liberating the spores as pollen grains. The fully developed pollen grains are tetrahedral with a thick exine and a thin delicate intine. The exine is not developed at the four corners of the tetrahedron, where thin areas represent weak spots. A large tube nucleus and a small generative cell lying towards one side are present at the shedding stage (Figs. 33 and 34).

In the mature anther the epidermis is usually torn asunder and the endothecium, with well-developed fibrillar thickenings, is therefore exposed externally to a large extent. The middle layers are very much crushed and



FIGS. 36-42.—Fig. 36. Nucellar primordium showing a group of potential archesporial cells, of which one marked *x* develops further.  $\times 1350$ . Fig. 37. Young ovule showing the free integuments. A large parietal tissue is seen above the developing megaspore.

× 200. Fig. 38. Two-nucleate embryo-sac. × 900. Fig. 39. Fully developed embryo-sac showing the parietal tissue forming the glandular apex of the nucellus. × 630. Fig. 40. Double fertilization. × 120. Figs. 40a & 40b. Fertilization and triple fusion respectively enlarged from Fig. 40. × 1350. Fig. 41. Embryo-sac after fertilization showing remnants of nucellar cells in the general cytoplasm. × 200. Fig. 42. Embryo-sac showing free endosperm nuclei. The lower end of the sac is forming processes into the nutritive tissue. × 120.

the tapetum is completely broken down (Fig. 35). Dehiscence of the anther is similar to that in *Grevillea robusta* Cunn. described by Brough (1933).

#### *The Ovule and the Development of the Embryo-sac.*

The ovary contains two atropous pendulous ovules, each of which arises as a nucellar primordium (Fig. 36). In the latter are soon evident a number of large cells with conspicuous nuclei. Along with these are also found other nucellar cells, but less conspicuous and with smaller nuclei. The larger cells form a group of potential archesporial cells, of which only one develops further. Though it is usually difficult to pick out this particular cell from the others of the same nature, it may be recognised to some extent by its median position in the nucellus and the large nucleus, which is usually a little in advance of the nuclei in the other cells ( $x$  in Fig. 36). Ballantine (1909) evidently refers to the same condition when he states that in *Protea Lepidocarpon* R. Br. "A small group of large cells situated below the hypodermal layer includes one which becomes the megaspore mother cell". In *Grevillea robusta* Cunn., on the other hand, a definite archesporial cell is always evident even in the early stages (Brough, 1933; Kausik, 1938 b). The division of the archesporial cell into the primary parietal cell and the megaspore mother cell could not be traced, but it may be inferred that such is the case by the presence of a large mass of parietal tissue (Fig. 37), which forms in later stages the characteristic glandular apex (Fig. 39) of the nucellus.

The integuments arise late; for instance, when the group of potential archesporial cells is evident in the nucellus, there is absolutely no indication of the origin of the integuments (Fig. 36). After they have been formed, they grow rather slowly all round the nucellus, but fail to close over completely at the apex of the nucellus even when the embryo-sac is fully developed and is ready for fertilization. The apex of the nucellus, therefore, lies exposed until fertilization, after which the integuments begin to grow again and invest the nucellus completely.

Both the integuments are made up of three or four layers of cells. The cells of the outer integument contain plenty of tannin (Fig. 20). The integuments are completely free from each other, as also the inner from the nucellus up to its very base.

The apex of the nucellus is glandular and is exposed before fertilization. This glandular tissue seems to be derived from the parietal cells, and also in part from the epidermis, and takes part in guiding the pollen tubes towards the embryo-sac. It also perhaps serves as a source of nutrition for the micropylar end of the embryo-sac, for, it persists even after fertilization and begins to break down only in the maturing seed containing a large embryo (Fig. 49).

The chalazal region of the ovule contains groups of tannin-filled cells (Fig. 20), which become contiguous as the seed begins to develop and form an extensive pad of cells at the chalaza (Figs. 44 and 47). Immediately above this pad the chalaza also contains a zone of great meristematic activity, where the cells divide rapidly, particularly after fertilization, and form a large nutritive tissue (Fig. 44). A similar condition is also met with in *Grevillea robusta* Cunn. (Brough, 1933 ; Kausik, 1938 b), but in *Protea Lepidocarpon* R. Br., Ballantine (1909) states that it "remains active until about the time of fertilization". This tissue is destroyed later by the embryo-sac (Figs. 42, 43 and 47), when the endosperm and the embryo are formed.

The development of the megaspores and the formation of the embryo-sac proceed along normal lines (Figs. 37 to 39). The fully developed embryo-sac contains two synergids with broad posterior ends, the egg, two polar nuclei remaining free but close together till fertilization, and lastly, three distinct antipodal cells (Fig. 39). The micropylar end of the embryo-sac is broad and rounded, while the antipodal end is narrow and pointed. In the formation and growth of the embryo-sac, the surrounding nucellar cells are crushed to a large extent. In these nucellar cells may be recognised in earlier stages, a few elongated ones with very little contents. These are evidently the non-functioning archesporial cells.

#### *Fertilization.*

The pollen tubes, on coming in contact with the exposed apex of the nucellus, pierce through the latter and finally enter the embryo-sac. In this, only one synergid seems to be destroyed (Fig. 40). The first male nucleus fuses with the egg nucleus, while the second unites with the two polar nuclei somewhere in the centre of the embryo-sac (Figs. 40, 40 a and 40 b). The stages showing the actual fusion of the nuclei were not available.

#### *Post-Fertilization Changes in the Ovule.*

After fertilization several interesting changes take place in the ovule. The integuments, which were originally incomplete at the apex of the nucellus, begin to grow again and form a complete covering (Fig. 47). The inner integument does not show any increase in the number of layers, while the

outer one becomes very thick and consists of many layers of cells storing plenty of tannin (Fig. 47). In *Protea Lepidocarpon* R. Br. (Ballantine, 1909) however, "the inner integument becomes very long and several cells thick, the outer remaining only two cells thick". With the increase in the thickness of the outer integument in *Macadamia* F. Muell., vascular strands arise, which are formed by tracks of elongated cells developing in the outer integument after fertilization. In the fully developed seed, the outer integument forms a hard stony covering.

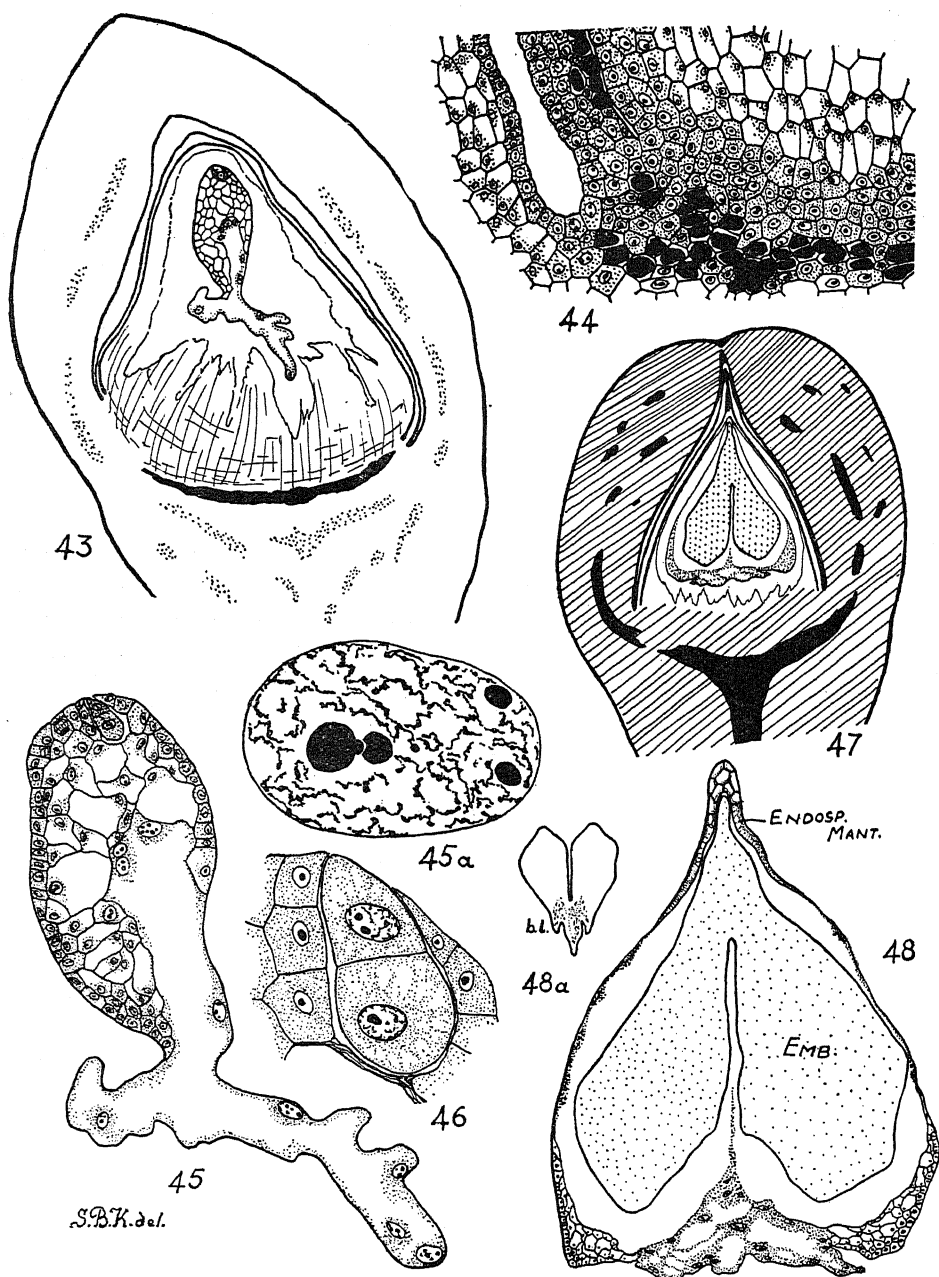
With the growth of the integuments, the nucellus also becomes more and more massive, especially at the region of the chalaza, where the large mass of nutritive tissue is developed. This is due to the activity of the chalazal meristematic cells. The entire mass of nucellus is completely used up when the endosperm and the embryo begin to develop (Figs. 42, 43 and 47).

#### *Endosperm.*

The primary endosperm nucleus undergoes repeated free nuclear divisions, by which groups of nuclei become scattered in the embryo-sac (Figs. 41 and 42). With the formation of these nuclei, the embryo-sac grows enormously in size and encroaching on the surrounding nucellus, becomes irregular in outline (Figs. 42, 43 and 45). The surrounding nucellar cells are practically dissolved away and appear in earlier stages as almost unrecognizable remnants floating in the general cytoplasmic mass of the embryo-sac (Fig. 41).

After a number of free nuclei are formed in the embryo-sac, wall formation begins in the formation of the endosperm tissue. In this, only the upper half of the embryo-sac takes part, while the lower remains free nucleate and forms irregular processes for absorbing materials (Figs. 43 and 45). In the upper half two distinct regions may be recognized. Towards the periphery the endosperm cells are small, compact and have rich contents, while those towards the inside are larger with less contents, irregular and loosely arranged (Fig. 45). The peripheral cells are in immediate contact with the surrounding nucellus and so take part in absorbing materials at the sides of the embryo-sac. These cells are comparable to the absorbing cells described in *Grevillea robusta* Cunn. (Kausik, 1938 a; 1938 b).

The lower half of the embryo-sac, where the nuclei remain free without forming cells, grows irregularly towards the nutritive tissue of the chalaza in the form of several processes (Figs. 42, 43 and 45). These processes, containing prominent nuclei which have a very high chromatin content, are of the nature of haustorial structures extending into and breaking down the nutritive tissue here and there (Figs. 43 and 47). Thus the entire mass of the



FIGS. 43–48.—Fig. 43. Longitudinal section of developing seed showing the embryo-sac with the upper cellular and lower free-nucleate endosperm. The lower portion is forming processes for absorption. Note the destruction of the nutritive tissue of the chalaza by the invading processes.  $\times 25$ . Fig. 44. Chalazal region of ovule showing tannin cells and

meristematic tissue. Portions of integuments are also seen.  $\times 400$ . Fig. 45. Embryo-sac from Fig. 43 enlarged to show details of endosperm. A two-celled embryo is seen.  $\times 80$ . Fig. 45a. A single free endosperm nucleus from Fig. 45 to show the high chromatin content.  $\times 1350$ . Fig. 46. Two-celled embryo.  $\times 400$ . Fig. 47. Longitudinal section of seed showing the massive outer integument containing tannin cells (hatched). Fig. 48. Embryo and remnants of endosperm forming the *Endosperm Mantle* for the radicle. Fig. 48a. Basal lobes of cotyledon in the embryo.

chalazal nutritive tissue is destroyed completely, as also the nucellus at the sides of the embryo-sac, so that a large cavity is formed within the integument. In this cavity the large embryo lies freely in later stages. The lower portion of the embryo-sac forming the processes is identical with the more definitely organised *Vermiform appendage* of the endosperm described in *Grevillea robusta* Cunn. (Kausik, 1938 a ; 1938 b).

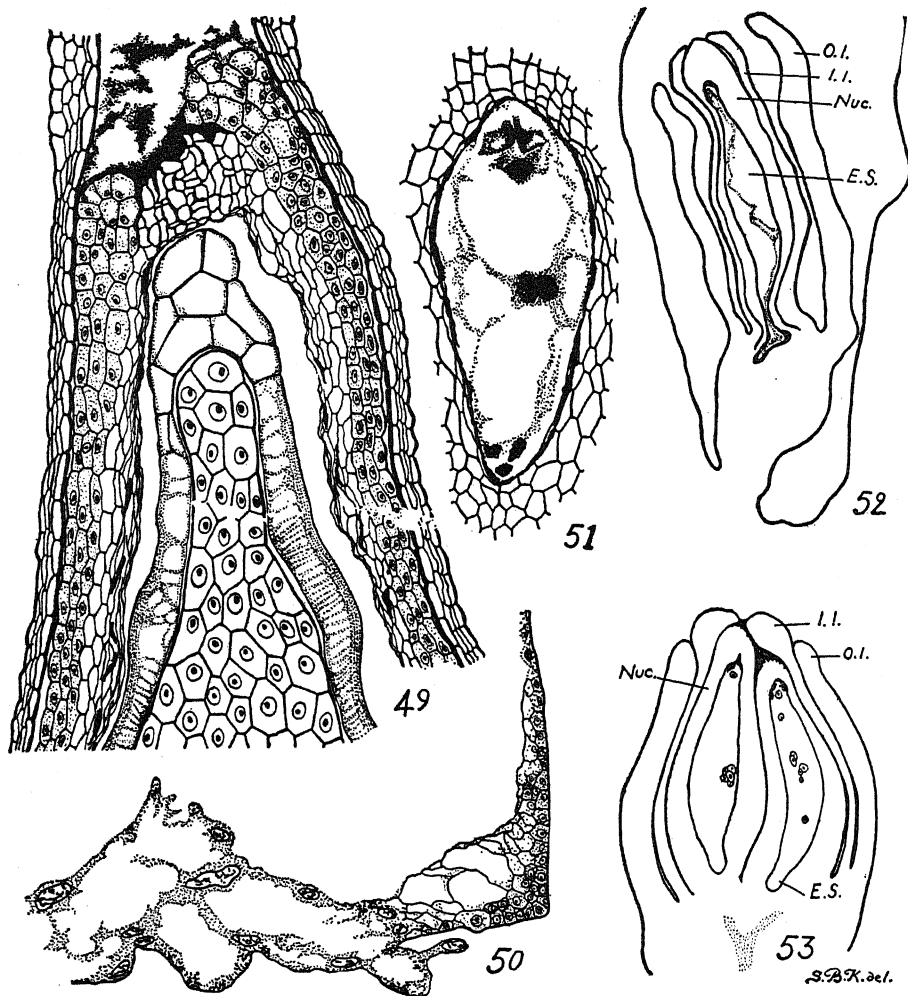
As the embryo begins to develop, the endosperm is in its turn destroyed (Figs. 47 and 48) so that the mature seed is non-endospermic. A few cells however persist at the region of the micropyle and extending down a little (Figs. 48 and 49) and also at the lower portions of the embryo-sac (Figs. 48 and 50). The persisting endosperm cells at the micropyle are devoid of contents and form an almost structureless cap or sheath (except at the very tip where the cell outlines may be recognized) for the radicle of the embryo, the *Endosperm Mantle* (Figs. 48 and 49).

#### *Embryo.*

The fertilised egg remains without dividing for a long time. For instance, when the endosperm is already a mass of cells, with the distinction into the upper and lower regions (Fig. 45), there is only a two-celled embryo (Fig. 46). Further stages in the development of the embryo could not be studied, as most of the ovules seem to degenerate after fertilization. Perhaps embryogeny is very similar to that in *Grevillea robusta* Cunn. (Brough, 1933 ; Kausik, 1938 a and 1938 b). The fully developed embryo (Fig. 48) consists of two massive cotyledons, each with two basal lobes (Fig. 48 a), a short hypocotyl and a radicle provided with a pointed root-cap. The tip of the radicle fits into the long and narrow micropyle formed by the growth of the inner integument after fertilization (Fig. 49).

#### *The Seed.*

Of the two ovules of an ovary, only one develops successfully into the seed, while the other is suppressed. The mature seed has a very thick outer coat (Fig. 47), which becomes extremely hard and stony. This is formed by the outer integument. Within this hard coat are the thin and crushed inner integument, a few surviving layers of the peripheral portions of the nucellus and the remnants of the endosperm. All these are pressed firmly



FIGS. 49-53.—Fig. 49. Portion from Fig. 48 enlarged to show the inner integument, disorganising apex of the nucellus and the *Endosperm Mantle* forming the sheath for radicle.  $\times 280$ . Fig. 50. Remnants of endosperm at the lower end of the embryo-sac, enlarged from Fig. 48.  $\times 80$ . Fig. 51. Degenerating embryo-sac.  $\times 630$ . Fig. 52. Ovule degenerating after fertilization showing the collapsed embryo-sac.  $\times 80$ . Fig. 53. An abnormal ovule showing two nucelli and common inner and outer integuments.

together and fusing with the outer hard coat of the seed, form a smooth and shining internal lining for the outer integument. Inside the seed there is a large cavity containing the massive embryo.

#### *Degenerations and Abnormality.*

During the course of this investigation, several cases of degenerations and one instance of abnormality were noticed. The ovules seem to degenerate

almost at any stage of development, both before and after fertilization. Several cases of degenerating ovules containing young and growing embryo-sacs, and also those with fully developed embryo-sacs ready for fertilization (Fig. 51), were met with.

Instances of degenerations of ovules after fertilization were more numerous. Many of the ovules containing embryo-sacs after fertilization, in some of which even a few free endosperm nuclei were seen, were found to have degenerated (Fig. 52). The embryo-sacs in these ovules are very narrow and crushed. The funiculi of the degenerating ovules are usually slightly longer than those of normal ones.

The single instance of abnormality consisted in the presence of two nucelli in an ovule, with common inner and outer integuments (Fig. 53). This abnormality has perhaps arisen by a splitting of the original nucellus. In each nucellus there is an embryo-sac showing remnants of pollen tube, the fertilized egg and a few free endosperm nuclei. The vascular tissue of the chalazal region of the ovule is forked into two limbs, each of which passes towards the base of an embryo-sac. Since this abnormal condition was met with only accidentally and in a solitary case, it is impossible to determine the fate of such ovules.

#### Conclusions.

The anatomy of the flower shows that the perianth lobes are of the nature of sepals, while the whorl of petals has disappeared altogether. It is therefore regarded that the Proteaceae are derived from dichlamydeous ancestors. A similar view is also held by Joshi (1936) with regard to the flowers of *Stellera chamæjasme* Linn., a member of the Thymelæaceae, with which order the Proteaceae are claimed to be related by several authors. There is however one difference; while the disc scale in the flower of *Stellera* Linn., is a much reduced part of the corolla, the scale in *Macadamia* F. Muell. has no such claim.

The presence of a single whorl of perianth is perhaps correlated to a large extent with the prevailing dry climate in which the members of the Proteaceae are generally found. The outer whorl of the flower, namely that of the sepals, has assumed such special and protective features by a dovetailing of the margins of the lobes that it forms not only an effective covering for the essential organs of the flower, but is also capable of becoming easily displaced by the pollinating agents at the time of pollination. The specialization of the outer whorl has resulted in a total elimination of the whorl of petals as the latter became less and less effective as a floral envelope. In the absence of cross-pollination, the single perianth whorl persists as a

"cap" and close or self-pollination is induced at least as a last measure. The topics relating to pollination and the phenomenon of "capping" in *Grevillea robusta* Cunn. have been discussed by Brough (1933) and his conclusions may also be applicable here.

In the structure of the ovary, a primitive condition of the carpel as visualised by Hunt (1937) in the form of an essentially three-lobed structure is perhaps indicated to some extent. Both in structure, as well as in anatomy, the Protead carpel, as revealed in *Macadamia* F. Muell., seems to have retained some of the features of such a primitive carpel.

Several features, which may be regarded as primitive, are also met with in the structure and development of the ovule. In comparing the ovule of *Myrica Gale* with the fossil seed *Trigonocarpus* Kershaw (1909) states: "The ovules of some of the Pteridosperms and many of the fossil Gymnosperms have been described as having a free nucellus.

"This character of a free nucellus in these older fossil seeds may indicate that the integument had not as yet become an integral part of the seed. A free nucellus, therefore, may be regarded as a primitive character which has been lost in the greater number of Angiosperms, where the integument and nucellus are fused together almost to the apex of the ovule."

It is interesting to note the presence of a free nucellus in *Macadamia* F. Muell., belonging as it does to a family considered primitive. But Benson and Welsford (1909), commenting on the work of Kershaw, remark that the presence of a free nucellus is "almost universal among Angiosperms".

The other features of the ovule regarded as primitive are: the apex of the nucellus which is exposed on account of an incomplete growth of the integuments before fertilization; the presence of abundant tannin cells in the floral parts, which seems to be characteristic of plants belonging to the primitive orders; the increase in the nucellar tissue due to the activity of meristematic cells; and the presence of vascular strands in the outer integument of the developing seed. This last is perhaps associated with the enormous increase in the thickness of the integument after fertilization and therefore, in response to a greater need for the supply of materials.

The causes underlying degenerations of the ovules at practically all stages are rather hard to determine. The more numerous instances of degenerations after fertilization suggest that the failure of cross-pollination, due both to the absence of the proper pollinating agents and to the scarcity of plants locally, is an important factor. Self-pollination does not seem to induce the formation of seeds to a great extent. On the other hand, the failure in seed production may be inherent in the members of the Proteaceæ,

for, Ballantine (1909) remarks : " In many of the South African representatives of the Proteaceæ only a small percentage of the flowers set seed. "

Summary.

The vascular anatomy of the flower shows that the perianth in *Macadamia ternifolia* F. Muell. is the whorl of sepals, while the corolla has completely disappeared. Thus a dichalmydeous ancestry is suggested for the Proteaceæ. The adnation of the stamens to the perianth is of recent origin.

The nature of the carpel is discussed in the light of the interpretation of Hunt (1937) regarding the origin of the modern Angiospermic carpel.

The structure of the anther is described. The pollen grains contain a tube nucleus and a small generative cell at the shedding stage.

The nature and development of the ovule are pointed out. The embryo-sac develops along normal lines.

Fertilization and changes in the ovule after fertilization are described. The nature of endosperm, some stages in the development of the embryo and the parts of the mature seed are dealt with.

Several instances of degenerations of ovules and one instance of an abnormal ovule with two nucelli and common inner and outer integuments are recorded.

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# STUDIES ON THE CAUDAL AUTOTOMY AND REGENERATION IN *MABUYA DISSIMILIS* HALLOWELL.\*

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## 1. Introduction.

THE phenomena of caudal autotomy and regeneration in Lizards have attracted the attention of many workers from time to time, and the salient features in this connection are fairly well known. Amongst the older workers, Müller (1852), Hyrtl (1853), Gegenbaur (1865), Fraisse (1885), Boulenger (1888), Werner (1892, 1896) and Tornier (1897) may be especially mentioned† while amongst the later ones, Misuri (1910), Woodland (1920), Slotopolsky (1922) and White (1925) have made notable contributions. Many workers have made a more or less intensive study of the anatomy of the regenerated part (Tytler, 1865; Brindley, 1894, 1898; Morgan, 1901; Annandale, 1904; Terni, 1922; Byerly, 1925; Guyenot, 1928; Marcucci, 1932, etc.); while others have dealt with such abnormalities as double or triple regenerated tails (Brindley, 1894, 1898; Fischer, 1907; Stuart, 1908; Graper, 1909; Gay, 1909; Ahl, 1927; Das, 1932, etc.). Hooker (1912) makes an interesting communication on the disposition of nerves in the regenerated tail; while Mahendra (1936) suggests that the presence of a Reissner's fibre in the caudal section of the spinal cord is correlated with the automatic movements of the autotomised piece after its separation from the body.

A study of the relevant literature, however, reveals that all the work with the exception of only a few papers (Hyrtl, 1853, on the vertebræ of various families; Alonzo, 1903, on *Gongylus*; Brindley, 1898, on *Mabuya*;

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\* Thesis approved as part of the requirements for the M.Sc. degree (1938) in the University of Agra.

† Hoffmann (*Kl. u. Ordn. d. Thier-Reichs*, 1890, 2, 470-75), gives a good résumé of the older work on this subject.

Fischer, 1907 on *Agama*; Woodland, 1920, on *Pygopus*),<sup>‡</sup> mainly concerns two families amongst Sauria, viz., *Lacertidæ* and *Gekkonidæ*; and that the other families showing such phenomena have not been studied in detail. The value of an all-round investigation of the remaining lacertilian families is clearly necessary to complete our knowledge and to enable us to draw phylogenetic conclusions. The present paper extends our knowledge in this connection to the family *Scincidæ*, which has only been imperfectly worked out by two previous workers (Alonzo, 1903 and Brindley, 1898). The skink, *Mabuya dissimilis* Hallowell, is a fossorial lizard widely distributed in the Northern India. According to Smith (1935), it is found in "Waziristan; Sibi district (Baluchistan); near Shikarpur (Sind); Ajmer (Rajputana); Salt Range, Rawalpindi, Rajanpur, Bhawalpur, Chamba (Punjab); Jubbulpore (C.P.); Hazaribagh, S.W. of Rajmahal (Bihar); Karharbari, Sahibganj, Boogoolah (Bengal)". It is a suitable subject for these investigations on account of its size and availability.

## 2. Material and Technique.

Over twenty specimens were used for this study, all of which were obtained alive from the jungles round about Agra. They were identified by my teacher Mr. Beni Charan Mahendra and confirmed by Dr. Malcolm A. Smith. They ranged in size from 5.3 to 9.3 cm. (length between snout and vent), and included representatives of both sexes.

For the osteological study, alizarin-KOH method of staining, as recommended by Mahendra (1936), was employed. The musculature was studied by careful dissections either with the naked eye or with the help of the dissecting microscope. For the gross and detailed study of the various tissues in the tail, transverse and longitudinal sections were either cut by hand and observed without staining, or they were prepared by microtechnique, the stain used being Mallory's triple stain (Peacock, 1935). The material for sectioning was decalcified either in 3 per cent. nitric acid in 70 per cent. Alcohol, or in the following mixture (Ranvier, *Lee's Vade Mecum*, 1928, p. 260):

100 c.c. cold saturated solution of Sodium Chloride in water.

100 c.c. water.

4 c.c. Hydrochloric acid.

Preparations were placed in this mixture, and 1 to 2 c.c. of hydrochloric acid were added daily until the material was softened.

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<sup>‡</sup> Woodland (1920) and Byerly (1925) deal with these phenomena in *Sphenodon punctatum*, and make it possible for us to compare the lacertilian features in this connection with those found in the Rhynchocephalia.

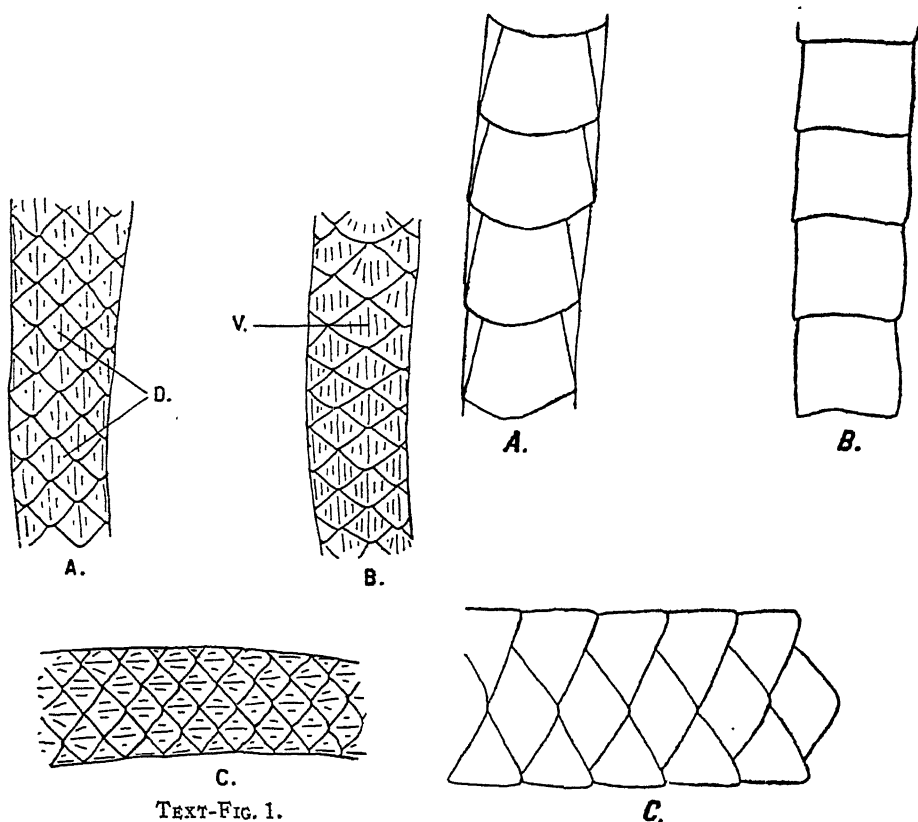
In order to determine whether the skeletal tube in the regenerated tail was cartilaginous or bony in nature, histological preparations were studied, as well as specimens were stained for cartilage according to Van Wijhe's methylen bluesolution (.25 grams of methylen blue in 100 c.c. of 70 per cent. alcohol with 1 per cent. of hydrochloric acid) and with alizarin solution.

### 3. *External Features in Relation to Caudal Autotomy.*

(a) *Normal Tail.*—The tail in *Mabuya dissimilis* is a long, strongly tapering structure, more or less oval (in cross section) with distinct lateral compression. It measures approximately as much as, or even more than the rest of the body (*i.e.*, the distance between snout and vent) and is covered with imbricate scales. If Smith (1935) is right in identifying Hora's *Mabuya hodgarti* (1927) with Hallowell's *Mabuya dissimilis* (1857), then Hora's statement that the species "possesses a prehensile tail" is open to doubt. I have not only scrutinised the anatomical structure of the tail, but have also kept several individuals alive under observation; in none of them was the tail found to be prehensile. Hora's view that the species probably is arboreal receives no confirmation from observations on living individuals. All the specimens kept by me in captivity were markedly burrowing in habit.

In the Gekkonid lizard *Hemidactylus flaviviridis* Rüppel (Woodland, 1920), the autotomy segments are clearly differentiated externally by the arrangement of the scales and the presence of annular lines of cleavage. In *Mabuya dissimilis*, however, the superficial segmentation corresponding to autotomy planes is altogether absent. There are no lateral projecting scales as in *Hemidactylus*, and the scale rows are arranged distinctly in an oblique manner, none being strictly transverse. This becomes clear when the tail is broken and the lepidosis of the two separated ends of the autotomised pieces carefully observed. Each autotomy segment here as well as in *Hemidactylus flaviviridis*, bears two transversely enlarged scales, one lying behind the other, on the ventral side. The tail shows such ventrals along its entire length, similar in appearance to the single ventral shields of snakes. The first autotomy plane lies somewhat behind the vent, the distance depending on the size of the animal to a considerable extent. The number of autotomy segments was counted in four specimens of *Mabuya dissimilis*, and varied from 42 to 46.

(b) *Regenerated Tail.*—In the normal tail of *Mabuya dissimilis*, the scalation shows an imbricate, oblique disposition, each row ending mid-ventrally in an enlarged ventral scale (Text-Fig. 1). The scalation of the regenerated tail (Text-Fig. 2) is similar to this, but differs from that of the normal tail in the fewer number of rows present (usually only four), and in the presence



TEXT-FIG. 1.

The scalation of the normal tail.  
 A.-dorsal view; B.-ventral view;  
 C.-lateral view; D.-mid-dorsal row of  
 scales; V.-ventrals.

C.

Text-Fig. 2.

The scalation of the regenerated tail.  
 A.-dorsal view; B.-ventral view;  
 C.-lateral view.

of an enlarged mid-dorsal in addition to the enlarged mid-ventral row. The transitional region between the normal and the regenerated parts has rather an irregular scalation.

The regenerated tail differs from the normal also in being much lighter coloured (rather pinkish) on account of the paucity of melanophores.

#### 4. General Arrangement of Tissues in the Tail.

In order to make out the disposition of the various tissues, I prepared and studied both hand-cut and microtome sections, transverse and longitudinal, both through the normal and the regenerated tails in *Mabuya dis-similis*. A comparison of such sections with those described for *Hemidactylus* (Woodland, 1920) and for *Lacerta* (White, 1925) shows some noteworthy differences.

A. Normal Tail.

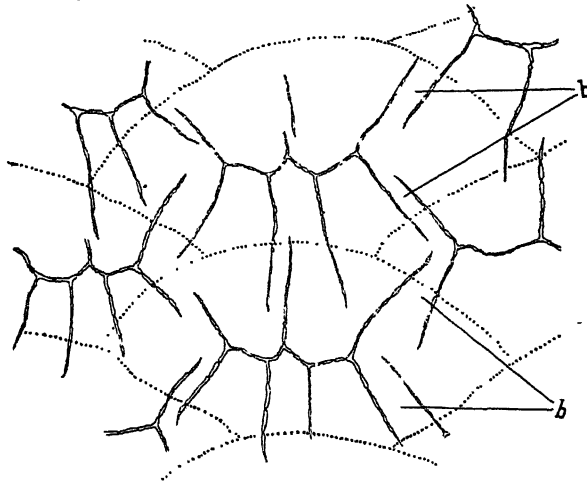
(i) *Epithelium*.—The horny scales and the bony scutes underlying them can both be readily made out. Each scale in transverse section, when observed under the high power of microscope, shows a composite structure, being made up of many short pieces joined together (Text-Fig. 3). The dermal



TEXT-FIG. 3.

Transverse section of a caudal scale, showing composite structure (under High Power).

scutes have the characteristic canals (Hewitt, 1929 ; Smith, 1935) ; but the alizarin-stained preparations (Text-Fig. 4) show that such scutes are not



TEXT-FIG. 4.

Arrangement of dorsal dermal scutes in the tail of *Mabuya dissimilis* (from an alizarin-stained preparation).

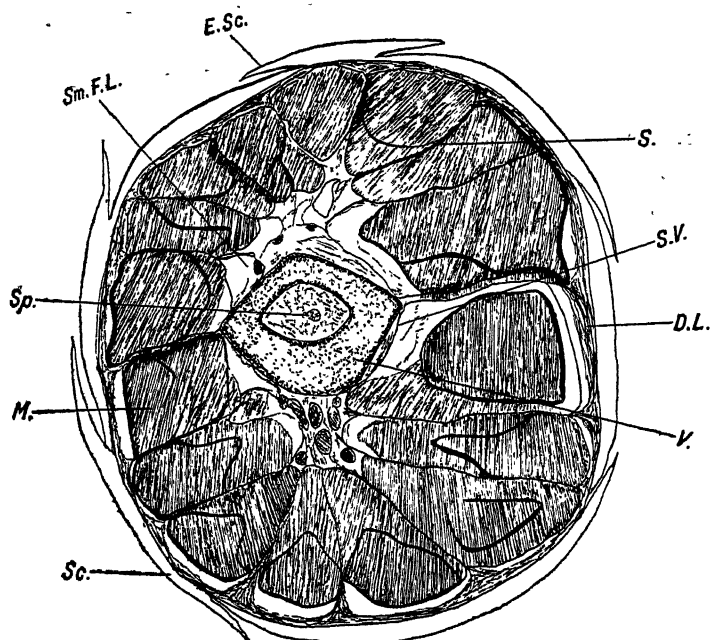
The dotted lines show the outer margins of the scales. *b.*—bridges interconnecting adjacent dermal scutes.

definitely marked off from each other at the boundary lines, but are interconnected by means of narrow bridges, thereby forming an extensive sub-epidermal armour. Such connections between the adjacent scutes, as far as I am aware, have not been previously recorded.

(ii) *Subcutaneous Fat-Layer*.—Woodland (1920) describes a subcutaneous fat-layer in *Hemidactylus flaviviridis* Rüppel, lying just below the skin, and "divided into cylindrical segments by lines of cleavage continuous with those of the skin".. White (1925) evidently did not find the corresponding layer

in *Lacerta vivipara*. In *Mabuya dissimilis*, there is a lining of horizontally-fibred connective tissue underlying the scutes and in all likelihood belonging to the dermis; under this is to be seen the covering membrane of the caudal muscles. A distinct subcutaneous fat-layer is absent.

(iii) *Caudal Muscles*.—These (Text-Fig. 5) lie below the dermis in sixteen bundles, of which the largest are situated at the sides of the vertebral



Text-Fig. 5.

Transverse section through the normal tail in *Mabuya dissimilis*.

*D.L.*—horizontally-fibred, subdermal lining; *E.Sc.*—overlapping edge of mid-dorsal scale; *M.*—caudal muscles; *S.*—intermuscular septum; *Sc.*—scale; *Sp.*—spinal cord; *Sm.F.L.*—submuscular fat layer; *S.V.*—sheath of connective-tissue around vertebra; *V.*—body of vertebra.

column and the smaller ones dorsal or ventral to it. The muscles are separated from each other by sixteen septa extending from below the dermis to the vertebræ, to which they depend at various places.

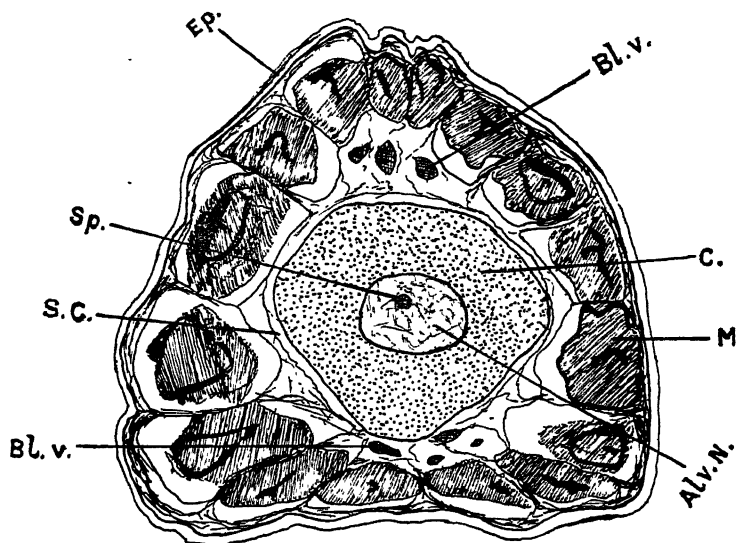
(iv) *Vertebral Column*.—Forming the innermost, axial skeleton of the tail is the vertebral column, which contains the spinal cord and its meninges. Immediately underneath the vertebræ are the lumph trunks, the caudal artery, the caudal vein and the nerves.

(v) *Sub-muscular Fat-layer*.—This layer is present between the caudal muscles and the vertebræ. The yellow fatty deposit is well seen in dissections and in the end-on aspects of the autotomised pieces, although it

disappears in microtome sections, probably on account of the fat having been dissolved away by xylol. This layer appears to be better developed lateral to the vertebræ. There are four bands of fatty tissues, two on each side, one of these being dorsal, and the other ventral, to the transverse processes of the vertebra.

### B. Regenerated Tail.

Transverse sections of the regenerated tail (Text-Fig. 6) resemble those of the normal one more in *Mabuya* than either in *Hemidactylus* or in *Lacerta*. Underneath the skin, which is deficient in pigment, is the usual connective-



TEXT-FIG. 6.

Transverse section through the regenerated tail in *Mabuya dissimilis*.

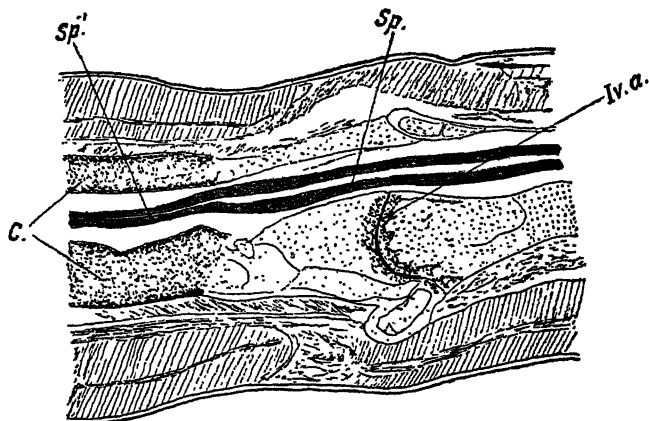
*Bl.v.*—blood vessels ; *Ep.*—epithelium ; *C.*—cartilaginous tube ; *Alv.N.*—alveolar net-work in the lumen of the cartilaginous tube ; *M.*—caudal muscles ; *S.C.*—sheath surrounding the cartilaginous tube ; *Sp.*—extension of the spinal cord.

tissue layer described above, and below this are the caudal muscles arranged into sixteen bundles like those in a normal tail. The sub-muscular fat-layer is present, surrounding the axial skeleton, and the caudal vein and artery can be made out almost in the same position as in a normal tail, although the hæmal arches are absent here.

The main differences from the original tail concern the axial skeleton and its contents, and are as follows :

(i) *Axial Section.*—In place of the long vertebral column there is a cartilaginous tube, roughly round in transverse section and surrounded by a sheath of fibrous tissue. This sheath is connected to the subcutaneous

connective-tissue lining by means of inter-muscular septa. An examination of median longitudinal sections shows that the lumen of the cartilaginous tube (Text-Fig. 7) is an extension of the neural space inside the original vertebral column of the tail, and that both the upper and the lower parts of the



TEXT-FIG. 7.

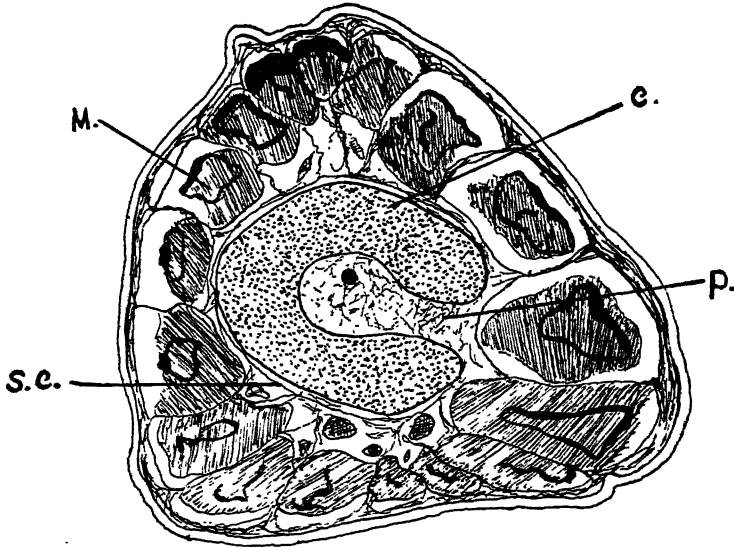
Longitudinal section through the tail of *Mabuya dissimilis*, showing the region of regeneration.

*Iv.a.*—inter-vertebral articulation, showing the two vertebrae firmly joined together. Other letters as in previous figures.

wall of the tube are almost equally developed. Histologically, the tube consists of innumerable cartilage-capsules rather closely packed together so that the matrix is not very considerably developed, each capsule having one or several cartilage cells, more or less shrunk away from its periphery. Both towards the outer and inner aspects of the cartilaginous tube the cells are smaller and more compactly arranged together. Externally the tube is surrounded by a delicate sheath (*perichondrium*). In *Hemidactylus*, Woodland (1920, Fig. 4 C) distinguishes a central uncalcified portion from a peripheral calcified one. In *Mabuya*, however, I do not find any such distinction, the peripheral and central portions being similarly constituted histologically; it is also significant that the whole cartilaginous tube takes up the alizarin stain uniformly. The cartilaginous tube does not show autotomy segmentation, nor does it have the various processes characteristic of vertebrae.

According to Powell White (1915, 1925), the cartilaginous tube in *Lacerta viridis* has occasional perforations for the passing in of blood vessels, etc. Woodland (1920) fails to find such perforations in the fully regenerated tail of *Hemidactylus*, but reports on their frequent occurrence in the young growing tube. My own transverse sections of the regenerated tail of *Mabuya*

*dissimilis* (Text-Fig. 8), show the presence of large openings placed ventrolaterally, and I find that the tube is rather flattened at this place. The openings are apparently covered over by the sheath of the cartilaginous tube.



TEXT-FIG. 8.

Transverse section through the regenerated tail of *Mabuya dissimilis*, showing a perforation (*P*) in the cartilaginous tube (*c*).

(ii) *Contents of the Cartilaginous Tube.*—The cartilaginous tube is lined internally by a delicate membrane, and it contains a prolongation of the spinal cord, some minute nerves and an alveolar net-work of non-cellular substance, interspersed with blood vessels and cells here and there. Both Woodland and White describe pigment cells also in this space, but as far as *Mabuya* is concerned, I find them absent. The contents of the cartilaginous tube show a remarkable similarity to the marrow region inside long bones.

##### 5. Caudal Vertebrae and their Relation to Autotomy.

In *Mabuya dissimilis*, contrary to what we find in other lizards, the main bodies of the two sacral vertebrae are almost wholly fused together, the zygapophyseal articulation between them is absent, and the centra have practically become one complete structure. The dual nature of these vertebrae is evident on account of the presence of paired transverse processes on each side and the occurrence of separate neural spines; yet the formation of what we may call a synsacrum is a clear phenomenon and even the transverse processes are confluent distally. The presence of fused sacral vertebrae in *Mabuya* is a noteworthy feature, as it forms exception to the general

condition described for Sauria. According to Sedgwick (1905, p. 336), the two sacral vertebræ in lizards "are not ankylosed, but they are united by strong ligaments".

After the sacral vertebræ, the two anterior caudal vertebræ (which are pre-anal in position) can be differentiated from the others by the absence of chevron bones. The transverse processes of the anterior caudal vertebræ are directed outwards and backwards, are stouter than those of the lumbar vertebræ and are flattened dorso-ventrally. The posterior caudal vertebræ, on the other hand, have these processes extremely minute and short, while the neural spines and the chevron bones are fairly elongate. This reduction of the transverse processes seems to be correlated with the distinct lateral compression of the hinder portion of the tail, as compared to the anterior portion. As we go posteriorly, the caudal vertebræ become smaller and smaller and their processes get gradually more reduced, until towards the very tip of the tail the vertebræ are represented by the extremely delicate, rod-like pieces into which, thin fibre-like extension of the spinal cord projects. A normal tail consists of 35 to 50 vertebræ, and each vertebra on the whole corresponds to two ventral shields.

With the exception of a few vertebræ at the anteriormost region of the tail, the caudal vertebræ are characterised by the presence of a definite autotomy septum, corresponding to what has been described in other autotomous lizards. The inter-vertebral articulations between each centrum and the succeeding one are of the ball-and-socket arrangement, but they are inseparable on account of the presence of strong, ligamentous unions. Each autotomy plane divides the vertebra concerned in such a way that the posterior part alone bears the neural spine and the chevron bones.

There has been a divergence of opinion about the nature of the autotomy plane dividing the caudal vertebræ. Gadow (1901, p. 494) regards it as a cartilaginous septum, while Woodland (1920, p. 83) thinks that it simply consists of a sheet of non-cellular hyaline substance which is continuous with those separating the other tissues of adjacent segments. In *Mabuya dissimilis* I have failed to find the presence of any definite septum, whether cartilaginous or hyaline; I can only note that there is a clear split in the osseous tissue of the vertebra at the level of the autotomy plane.

#### 6. Caudal Musculature.

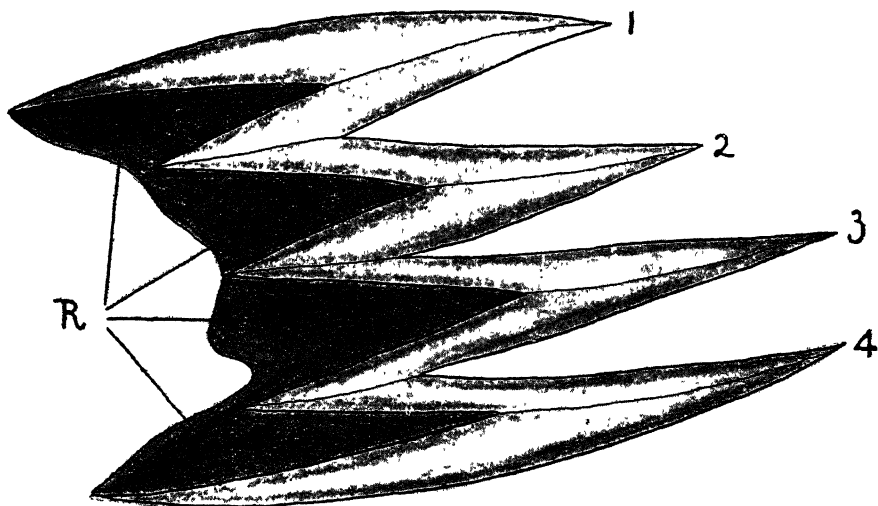
(a) *Normal Tail*.—In order to study the connections and disposition of the caudal flexor muscles, I have (like Woodland) examined not only transverse and longitudinal sections but also de-skinned whole preparations of autotomised pieces of the tail both from the anterior and the posterior aspects.

To bring out the essential differences of these muscles in *Mabuya* from those found in *Hemidactylus*, I shall first summarise the findings of Woodland.

In *Hemidactylus flaviviridis* there are eight muscle processes visible anteriorly in each autotomised segment (four dorsal and four ventral). Posteriorly, each of these processes is seen to bifurcate, the halves of adjacent processes uniting with each other except in the case of the dorsalmost and the ventralmost halves, which remain separate from each other. Thus, on the posterior side of the segment there are altogether ten points of termination of the muscles instead of eight anterior ones. The anterior processes of one autotomy segment dove-tail into the posterior ones of the segment preceding it, and so on.

In *Mabuya dissimilis*, the general disposition of the caudal musculature resembles that in *Hemidactylus* on the whole. However, the following important features, mainly differences, must be herein noted.

(1) The muscle processes (Text-Fig. 9) are arranged in two lateral series ; one right, the other left, each series consisting of four processes lying one



TEXT-FIG. 9.

Caudal musculature of a single autotomy segment from the right side.

1, 2, 3, 4—the four anteriorly projecting muscle processes ; 1', 2', 3', 4'—(R) four recesses for the accommodation of the anterior muscle processes of the autotomy segment behind.

below the other. This appears to be in correlation with the laterally compressed nature of the tail, and is in clear contrast to the arrangement as found in *Hemidactylus*, in which the tail is depressed dorso-ventrally. In the latter

lizard the eight processes are arranged into a dorsal and a ventral series, each series consisting of four.

(2) Each autotomy segment is distinctly telescoped into the segment behind it, and the plane of autotomy is thus very oblique and zigzag. In *Hemidactylus*, on the other hand, it is more or less straight and transverse.

(3) Transverse sections leave no doubt that there are sixteen bundles of muscles, each being ensheathed separately from the other. The adjacent muscles are united together, two by two, to form processes both anteriorly and posteriorly.

(4) As in *Hemidactylus* the adjacent dorsalmost and the ventralmost halves of the anteriorly directed processes remain separate from each other posteriorly, there being eight points of termination on the front-aspect of the autotomy segments and ten on the hind. These halves are more developed than the others. The other processes, which are laterally disposed, are hardly much prominent and are in the form of slight ridges, bounding scallop-like recesses for the accommodation of the anterior processes of the succeeding segment.

(b) *Regenerated Tail*.—The musculature of the regenerated tail corresponds to that of the normal in the presence of sixteen muscle bundles (Text-Fig. 6), clearly visible in transverse sections. The superficial examination of 'de-skinned' preparations, however, does not make it possible to distinguish the dove-tailing of processes as found in the normal tail. Yet it is important to notice that the regenerated tail can also be broken into pieces by vigorous pulling, and that here also as in a normal tail, there are eight elongate projecting processes on the anterior aspect of the hinder broken-off piece, which are lodged in eight cavities on the posterior aspect of the anterior one. Thus in all likelihood, there is a segmentation of the musculature even in the regenerated tail corresponding to that found in the normal. The extremely compact joining of the muscle pieces, however, stands in the way of autotomy within the regenerated piece and gives the appearance of unsegmented musculature running from the point of break to the tip of the newly-developed tail (cf. Woodland, 1920, p. 85).

## 7. Summary.

The following résumé brings together the more important features discovered during the course of this work :

(1) The lepidosis of the regenerated tail differs from that of the normal in the fewer number of scale rows and in the presence of the enlarged mid-dorsals in addition to the enlarged mid-ventrals. The normal tail is not segmented externally in correspondence to autotomy planes.

(2) The regenerated tail is lighter-coloured (*i.e.*, more or less pinkish in appearance) and is poor in melanophores.

(3) The general arrangement of the tissues in the tail of *Mabuya* is similar to that described for *Hemidactylus*, but the subcutaneous fat-layer is entirely absent.

(4) The dermal scutes show inter-connecting bridges and thus go to form an extensive body armour. This feature has not been so far noted by previous authors.

(5) The regenerated tail has also sixteen muscle bundles like the normal.

(6) The cartilaginous tube in the regenerated tail in *Mabuya* does not show any distinction between a central (uncalcified) and a peripheral (calcified) portion, as discovered in *Hemidactylus*.

(7) The cartilaginous tube has occasional large perforations.

(8) The contents of the cartilaginous tube show histologically a similarity to bone-marrow, and do not show any pigment cells.

(9) The sacral vertebræ in *Mabuya* are fused together to form a synsacrum. The anterior two caudal vertebræ have no chevron bones. The inter-central articulations between the caudal vertebrae are inseparable on account of strong ligamentous unions, and the vertebræ are divided by an autotomy plane, which is neither cartilaginous nor hyaline.

(10) The muscle processes are arranged in two lateral series, each consisting of four.

(11) Each autotomy segment is telescoped into the one behind it. There is no *distinct* autotomy septum in *Mabuya* as in other lizards—there being only a clear split in the osseous tissue of the vertebræ concerned.

(12) The breaking of the regenerated tail into pieces by vigorous pulling indicates that the caudal muscles here also are arranged in a "dove-tailing" manner. The number of muscle bundles is the same as that found in the normal tail.

#### 8. Acknowledgments.

The problem was suggested to me by my teacher, Mr. Beni Charan Mahendra under whose kind supervision I have had the privilege of working it out. I am much grateful to him for his continuous, painstaking assistance and guidance in this work. I am also indebted to Dr. Malcolm A. Smith (British Museum, London) for the identification of the species, to Prof. L. P. Mathur for the facilities enjoyed by me in the Zoological Laboratory of St. John's College, and to Dr. B. K. Das for his friendly criticisms and many valuable suggestions.

*Post-script.*

Following a suggestion of Mr. Mahendra, it was my intention to extend the present paper—before publication—by the addition of an account of the vascular and nervous systems of the original and regenerated tails, the rôle of the Reissner's fibre in autotomy, experiments on the processes of autotomy and regeneration, and the development of the caudal musculature. My recent appointment in the Forest Department, however, makes it impossible for me to continue the studies. I hope that somebody else in the Department will soon take it up.

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\* This paper—an excellent contribution to the subject, though written without knowledge of Woodland's and some other authors' works—contains a few references not included in the present Bibliography. The reader is, therefore, referred to it.

# MOLDS OF THE PUNJAB—I.

## The Aspergilli.

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AND

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IN this series it is intended to publish the molds of the Punjab. They are of great economic importance because of their enzymes and toxins. "They are a cause of spoilage of foodstuffs and other organic products of industry, they play an important part in the circulation of the elements of organic matter in nature (and therefore in maintaining of soil fertility), they bring about chemical reactions of value to man (and are therefore important as industrial ferments) and they produce disease in man and animals" (Henrici, 1930).

In this paper, the Aspergilli isolated and studied by the senior author and his pupils have been listed. Descriptions of many of these have been published already. The descriptions of the rest of the species are given here, as well as illustrations of most of the species studied in this laboratory. Also all Aspergilli, so far noted in India, have been listed here. This, it is hoped, will be of help to workers on Aspergilli in India. Thom and Church's (1926) method of description has been followed generally.

### *Descriptions of Species.*

#### 1. *Aspergillus fumigatus* Fresenius.

Thom & Church (1926), Butler (1917), Chaudhuri & Sachar (1932), Chaudhuri & Umar (1935), Finlow (1918), Galloway (1936) and Thakur & Norris (1928).

Isolated from soil and air (Fig. 1).

##### *A. fumigatus* Strain A (H7).

Chaudhuri & Sachar (1932).

Isolated from field, garden and alkali soils of Lahore.

##### *A. fumigatus* Strain B (H6).

Chaudhuri & Sachar (1932).

Isolated from humus soil, Lahore.

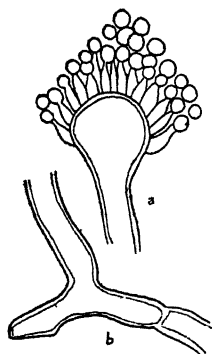


FIG. 1.—*A. fumigatus*.  
a—Head with sterigmata ;  
b—Foot-cell. Both  $\times 525$ .

2. *A. fumigatus* var. *tumescens* Blumentritt.

Thom & Church (1926), Galloway (1936), Thakur & Norris (1928),  
Chaudhuri & Umar (1935).

Isolated from soil and air.

On Czapek's solution agar, produces a dense buckled felt of mycelium. Conidiophores smooth,  $91.66\text{--}326.92\ \mu$  and in some cases  $570.36\ \mu$  long and  $3.4\text{--}6.37\ \mu$  thick, unseptate, gradually enlarging upwards into a flask-shaped vesicle,  $11.88\text{--}24.46\ \mu$  in diameter ; sterigmata in one series,  $5.1\text{--}7.4 \times 2.37\text{--}3.73\ \mu$  ; conidia smooth, globose,  $3.73\text{--}4.75\ \mu$  in diameter. Secondary heads from the outgrowth of sterigmata (Fig. 2).

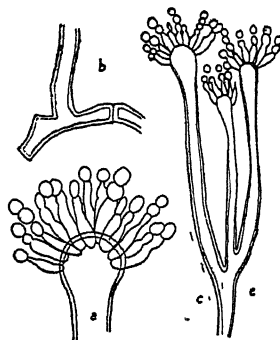


FIG. 2.—*A. fumigatus* var. *tumescens*.  
a—Head with sterigmata and spores.  $\times 450$  ;  
b—Foot-cell.  $\times 450$  ;  
c—Branched conidiophores.  $\times 245$ .

3. *A. polychromus* de Mello.

Thom & Church (1926), de Mello (1920).

Isolated from Petri-dish contamination.

4. *A. nidulans* (Eidam) Winter.

Thom & Church (1926), Chaudhuri (1932), Chaudhuri (1933),  
Chaudhuri & Sachar (1932), Chaudhuri & Umar (1935), Thakur &  
Norris (1928), Galloway (1936).

Isolated from soil, air and fruits of *Citrus*.

*A. nidulans* (H4).

Chaudhuri & Sachar (1932), Chaudhuri & Umar (1935).

Isolated from Lahore soil (Fig. 3).

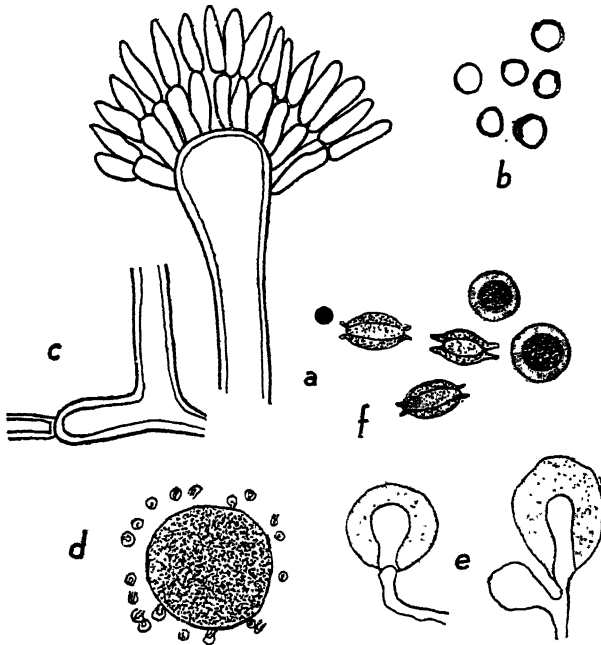


FIG. 3.—*A. nidulans* (H4).

*a*—Head with sterigmata.  $\times 1200$ .

*b*—Spores.  $\times 1200$ .

*c*—Foot-cell.  $\times 1200$ ;

*d*—Perithecia with hülle-cells.  $\times 120$ ;

*e*—Hülle-cells.  $\times 1200$ .

*f*—Ascospores.  $\times 750$ .

*A. nidulans* (B22).

Chaudhuri & Sachar (1932), Chaudhuri & Umar (1935).

Isolated from soil and air (Fig. 4).

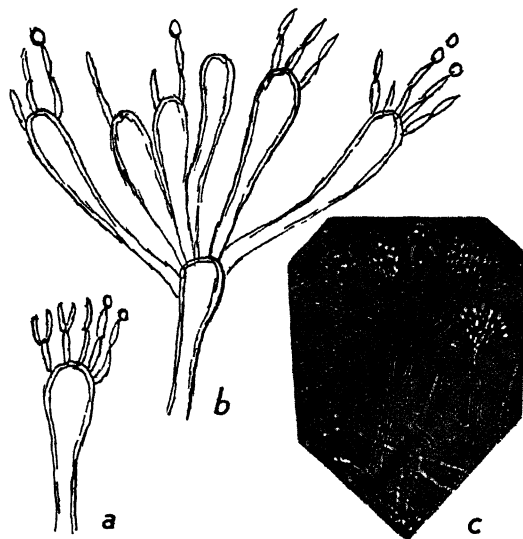


FIG. 4.—*A. nidulans* (B22).

a—Head with sterigmata.  $\times 650$  ;

b—Branching head.  $\times 650$ .

c—Photo-micrograph of same.

5. *A. versicolor* (Vuillemin) Tiraboschi.

Thom & Church (1926), Chaudhuri & Sachar (1932), Chaudhuri & Umar (1935).

Isolated from soil and air (Fig. 5).

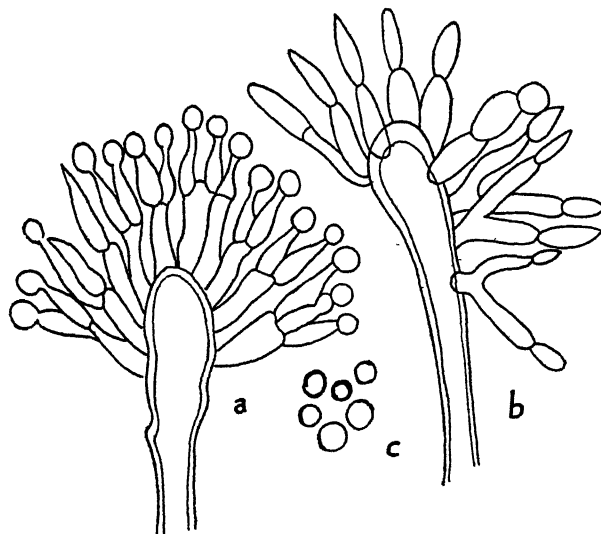


FIG. 5.—*A. versicolor*.

a—Head with sterigmata ;

b—Head with branching primary sterigmata ;

c—Spores. All  $\times 1200$ .

*A. versicolor*—Strain 3512.

Isolated from Petri-dish contamination.

Colonies white to pea-green and sage-green (Rdg. Pl. XLVII, b 29. G-G-Y); later light brownish olive (Rdg. Pl. XXX, k 21, O-VY); reverse colourless to reddish brown with age; surface velvety; conidiophores  $333.7\text{--}1340.1 \times 2.37\text{--}6.79\ \mu$ , unseptate, walls smooth, almost colourless; vesicles  $6.79\text{--}13.58\ \mu$  in diameter, usually flask-shaped, fertile on the upper two-third with radiating sterigmata in two series; primary sterigmata  $4.75\text{--}6.79\ \mu$ , secondary sterigmata  $1.5\text{--}2\ \mu \times 4.1\text{--}7.46\ \mu$ ; conidia globose,  $3.39\text{--}4.42\ \mu$  in diameter (Fig. 6).

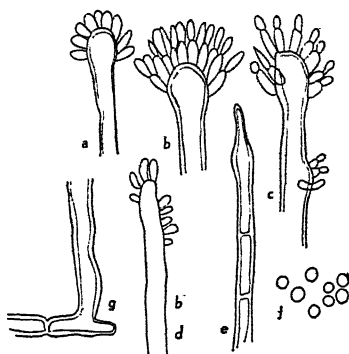


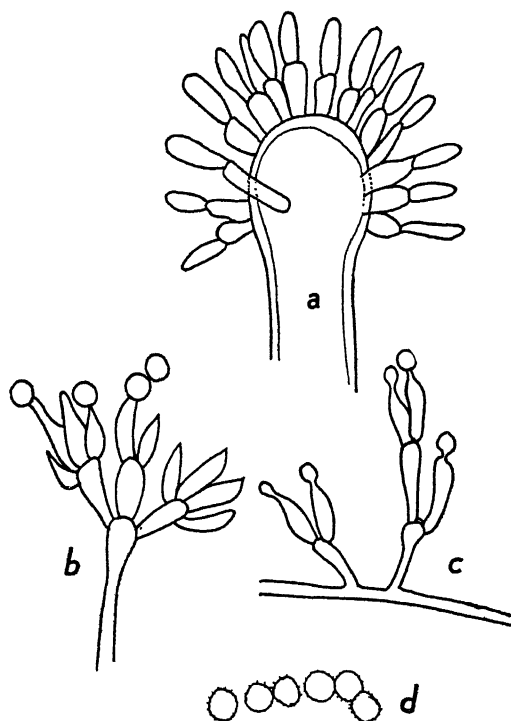
FIG. 6.—*A. versicolor* (Strain 3512).

- a*—Vesicle with primary sterigmata ;
- b*—Vesicle with primary and secondary sterigmata ;
- c, d, e*—Abnormal cases ;
- f*—Spores ;
- g*—Foot-cell. All  $\times 550$ .

6. *A. sydowi* (Bainier & Sartory) Thom & Church.

Thom & Church (1926), Chaudhuri & Sachar (1932), Chaudhuri & Umar (1935), Galloway (1936).

Isolated from soil and air (Fig. 7).

FIG. 7.—*A. sydowii*.

*a*—Vesicle with sterigmata ;

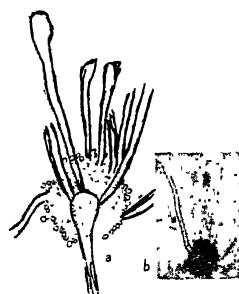
*b, c*—Heads with reduced penicillate conidial apparatus ;

*d*—Spores. All  $\times 1200$ .

7. *A. calypttratus* Oudemans.

Thom & Church (1926), Chaudhuri & Sachar (1932), Chaudhuri & Umar (1935).

Isolated from soil and air (Fig. 8).

FIG. 8.—*A. calypttratus*.

*a*—Primary head with nine secondary branches  $\times 100$  ;

*b*—Photomicrograph of same.

8. *A. terreus* Thom.

Thom & Church (1926), Chaudhuri & Sachar (1932), Chaudhuri & Umar (1935), Galloway (1936), Mason (1928).  
Isolated from soil and air (Fig. 9).

9. *A. fuscus* Amons.

Thom & Church (1926), Galloway (1936), Thakur & Norris (1928).  
Isolated from soil by Galloway.

10. *A. ustus* (Bainier) Thom & Church.

Thom & Church (1926), Galloway (1936).  
Isolated from soil by Galloway.

11. *A. humicola* Chaudhuri & Sachar.

Chaudhuri & Sachar (1932).  
Isolated from humus soil, Lahore (Fig. 10).

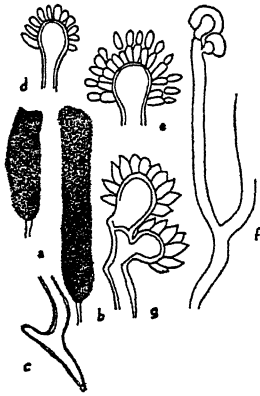


FIG. 9.—*A. terreus*.

a-b—Two complete heads.  $\times 75$  ;  
c—Foot-cell.  $\times 300$  ;  
d, e—Heads with sterigmata.  $\times 450$  ;  
f—Branching conidiophore.  $\times 240$  ;  
g—Apical portion of same.  $\times 550$ .

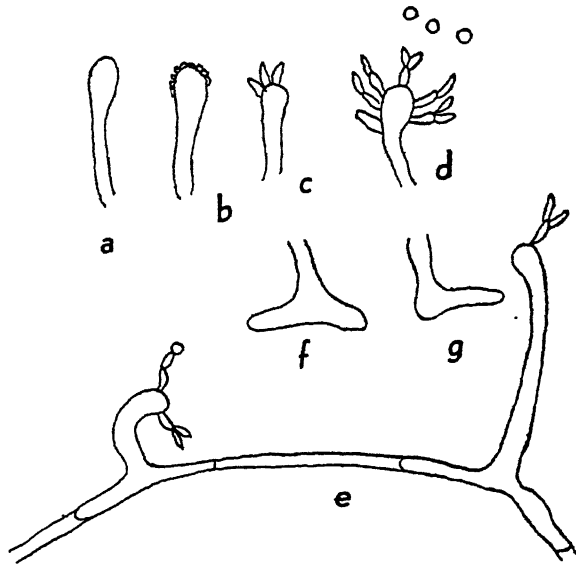


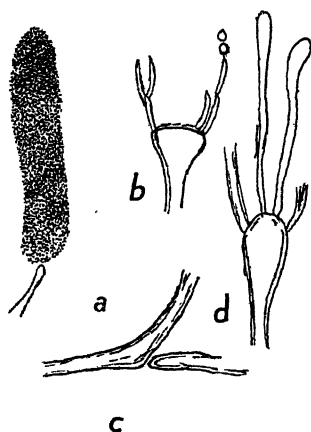
FIG. 10.—*A. humicola*.

a-d—Stages in development of sterigmata ;  
e—Conidiophores ;  
f, g—Foot-cells. All  $\times 550$ .

12. *A. flavipes* (Bainier & Sartory) Thom & Church.

Thom & Church (1926), Chaudhuri & Sachar (1932), Galloway (1936), Mahju (1933).

Isolated from soil, air and sheep's dung (Fig. 11).

FIG. 11.—*A. flavipes*.

- a—Complete head.  $\times 200$  ;  
 b—Head with sterigmata.  $\times 625$  ;  
 c—Foot-cell.  $\times 625$  ;  
 d—Abnormal branching.  $\times 625$ .

13. *A. candidus* Link.

Thom & Church (1926), Chaudhuri & Sachar (1932), Thakur & Norris (1928), de Mello & Carmo Vās (1921).

Isolated from soil and air.

14. *A. corroligena* Masee.

Thom & Church (1926).

On corolla of *Impatiens* sp., Manipur.

15. *A. niger* van Tieg.

Thom & Church (1926), Galloway (1936), Thakur & Norris (1928), Mason (1928), Hutchinson & Ayyar (1915), de Mello (1920), de Mello & Carmo Vās (1921), Tunstall (1924), Cooke (1872).

Isolated from soil and air, also within seeds of *Gossypium*, Dharwar ; in rice-beer fermentation ; in tea during fermentation.

*A. niger*-Al.

Chaudhuri & Umar (1935).

Isolated from Petri-dish contamination.

Colonies aniline black (Rdg. Pl. L, 69 r—Rm) ; produce abundant submerged mycelium with more or less yellow colour in the hyphæ but the substratum is almost colourless ; conidiophores unseptate, about 1 mm. long with thick bluish yellow walls ; vesicles mostly globose,  $20\cdot37$ – $57\cdot71\ \mu$  in diameter ; sterigmata in one series, compactly arranged along the entire length

of the vesicle,  $5.0-6.79 \times 2.37-3.14 \mu$ ; conidia oval and globose, rough,  $3.73-4.75 \mu$  thick and formed in columns (Fig. 12).

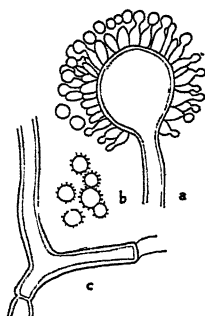


FIG. 12.—*A. niger*-A1.

a-Head with sterigmata ;  
b-Spores ;  
c-Foot-cell. All  $\times 450$ .

*A. niger*-A2.

Chaudhuri & Umar (1935)

Isolated from Petri-dish contaminations.

Colonies brown to black, reverse and substratum colourless; surface velvety with abundant submerged mycelium which is usually yellowish black in colour, vegetative hyphæ branched, septate,  $4.1-10.2 \mu$  thick; conidiophores arise from submerged hyphæ,  $212.4-758.7 \mu$  long, vesicles hemispherical;  $30.5-54.3 \mu$  in diameter; sterigmata  $5.71-10.2 \mu$ , closely borne in one series all over the vesicles; conidia globose, smooth,  $3.7-4.4 \mu$  in diameter, occurring in close radiating columns. Heads radiate (Fig. 13).

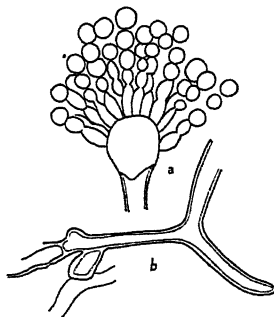


FIG. 13.—*A. niger*-A2.

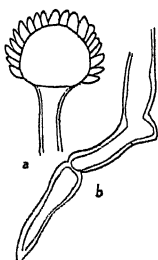
a-Head with sterigmata ;  
b-Foot-cell. Both  $\times 450$ .

16. *A. luchuensis* Inui.

Thom &amp; Church (1926), Chaudhuri &amp; Umar (1935).

Isolated from Petri-dish exposed to air.

Colonies purple brown to black with dirty yellow colour in the substratum; sterigmata only in one series; conidiophores 1-2.5 mm. in length with 10-15  $\mu$  in diameter, walls heavy, smooth, brownish, vesicles 20-30  $\mu$  in diameter; sterigmata 3.6  $\mu$ ; conidia 4-4.5  $\mu$  in diameter; finely roughened (Fig. 14).

FIG. 14.—*A. luchuensis*.*a*—Head with sterigmata;*b*—Foot-cell. Both  $\times 450$ .17. *A. (Sterigmatocystis) castanea* Patterson.

Thom &amp; Church (1926), Butler (1914).

On *Punica granatum* in India.18. *A. phaeocephalus* Durien & Montagne.

Thom &amp; Church (1926), Cooke (1878).

On roots of *Asparagus recemosus*, Madras.19. *A. ustilago* Beck.

Thom &amp; Church (1926), Butler &amp; Bisby (1931).

In pericarp of *Phyllanthus emblica*, Satpore Mountains.20. *A. sulphureus* (Fres.) Thom & Church.

Thom &amp; Church (1926), de Mello (1920).

Isolated from laboratory contamination.

21. *A. sachari* Chaudhuri.

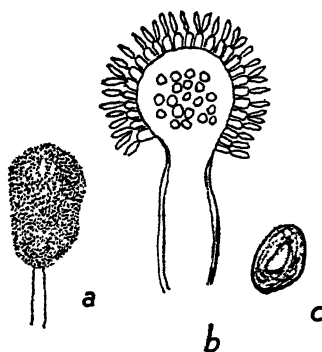
Chaudhuri &amp; Sachar (1932).

Isolated from Lahore soil (Fig. 15).

22. *A. ochraceous* Wilhelm.

Thom &amp; Church (1926), Galloway (1936).

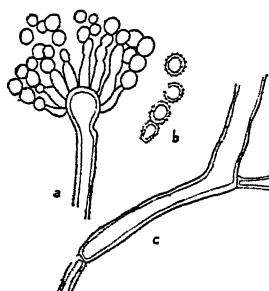
Isolated from soil by Galloway.

FIG. 15.—*A. sachari*.a—Complete head.  $\times 165$ .b—Head with sterigmata.  $\times 650$  ;c—Sclerotium.  $\times 6$ .23. *A. tamarii* Kita.

Tom & Church (1926), Galloway (1936), Mason (1928), Chaudhuri & Umar (1935).

Isolated from soil and air.

Colonies floccose at the centre but velvety towards the margin ; mycelium at first white, later pale yellow to yellowish-brown due to conidia formation, while in older colonies they become almost sepia coloured (Rdg. Pl. XXIX, 37, O-Y-m) ; reverse usually colourless but brown with age ; conidiophores arise from submerged hyphæ up to 1.2 mm. long and  $3.4-8-48\ \mu$  wide, increase in diameter towards the apex till abruptly pass into vesicles, walls rather thick but thinner at the base of vesicles ; vesicles globose or hemispherical,  $10.86-33.94\ \mu$  in diameter ; sterigmata in one series, closely compact, covering two-third to almost whole of the vesicles ;  $5.77-7.47 \times 2.04-3.39\ \mu$  ; conidia  $5.1-6.8\ \mu$ , mostly globose though a few pyriform, tuberculate specially at the distant end in the chain, rough. Sclerotia and perithecia not found ; all the heads bear sterigmata only in one series (Fig. 16).

FIG. 16.—*A. tamarii*.

a—Head with sterigmata ;

b—Spores ;

c—Foot-cell. All  $\times 450$ .

24. *A. oryzae* (Ahlburg) Cohn.  
Thom & Church (1936), de Mello (1920).  
Isolated from contaminated Petri-dish.
25. *A. flavus* Link.  
Thom & Church (1926), Thakur & Norris (1928), Mason (1928),  
Hutchinson and Ayyar (1915), Chaudhuri & Umar (1935).  
Isolated from soil and air and also on roots of *Polygala arillata*  
used for rice-beer brewing, Khasi Hills.

Colonies floccose, passing through several shades of yellowish green reach Saccardo's umber in old and dried colonies (Rdg. Pl. XXIX, 17. O-y-k); reverse and substratum generally colourless when young, becomes yellowish-brown to dark-brown with age; conidiophores 788-1500  $\mu$  long, 7.44-16.97  $\mu$  in diameter, broadening upwards with walls colourless, pitted or spiny, gradually enlarging to form a vesicle 10.2-30 or even 44  $\mu$  in diameter. Heads varying from small with a few chains of conidia to large stellate or columnar masses; sterigmata in one series, 6.79-10.18  $\times$  3-4  $\mu$ ; conidia pyriform to almost globose, somewhat colourless to yellow green, sometimes smooth, mostly appearing rough, 5.1-6.79  $\mu$  in diameter (Fig. 17).

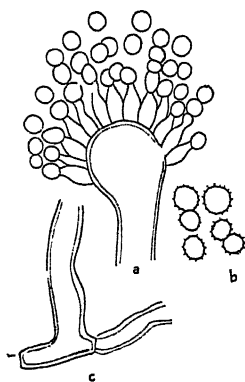


FIG. 17.—*A. flavus*.  
a—Head with sterigmata;  
b—Spores;  
c—Foot-cell. All  $\times$  550.

26. *A. herbariorum* Wiggers.  
Thom & Church (1926), de Mello (1920).  
Isolated from contaminated Petri-dishes.
27. *A. chevalieri* (Mangin) Thom & Church.  
Thom & Church (1926), Galloway (1936).  
Isolated from soil by Galloway.

28. *A. repens* (Corda) de Bary & Woronin.  
Thom & Church (1926), Thakur & Norris (1928).  
Isolated from soil.
29. *A. aguiari* de Mello.  
de Mello & Carmo Vās (1921).  
Isolated from urine, Goa.
30. *A. albicans* de Mello.  
de Mello & Carmo Vās (1921).  
Isolated from contaminated Petri-dish.
31. *A. ortæ* de Mello.  
de Mello & Carmo Vās (1921).  
Isolated from contaminated Petri-dish.

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## MOLDS OF THE PUNJAB—II.

### The *Penicillia*.

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Received June 22, 1938.

IN the second paper on the Molds of the Punjab, the *Penicillia* are grouped together. Out of 15 species of *Penicillium* and one of *Scopulariopsis* listed here, 11 have been studied in this laboratory. Remaining 5 have been included to make the list so far complete for all India. Of the forms studied here, descriptions of some have already been published by the author and his students ; descriptions of the rest of the species as well as illustration of most of the species noted from this laboratory are given to make the paper useful to workers on Indian *Penicillia*. Thom's (1930) method of description has been followed generally.

#### *Descriptions of Species.*

1. *Penicillium viridi-variens* Chaudhuri & Sachar.  
Chaudhuri & Sachar (1932).  
Isolated from soil (Fig. 1).

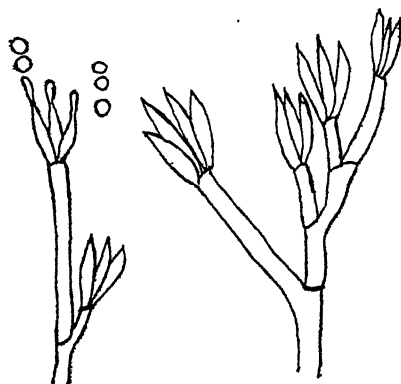


FIG. 1.—*P. viridi-variens*.

Mode of branching of conidiophore.  $\times 1080$ .

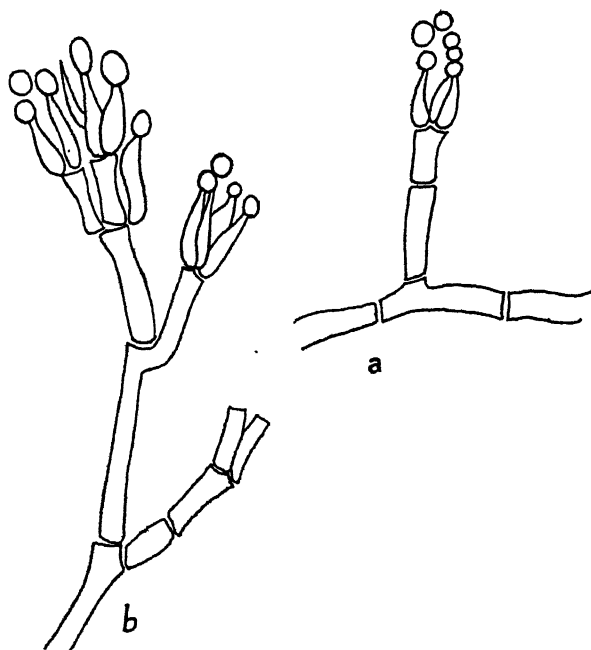
2. *P. glabrum* (Wehmer) Westling.  
Thom (1930), Thakur & Norris (1928).  
Isolated from soil.

3. *P. fellutanum* Biourge.

Thom (1930), Chaudhuri &amp; Umar (1935).

Isolated from Petri-dishes exposed to air.

Colonies upon Czapek's solution agar, narrowly growing with margin velvety and not over 100–200  $\mu$  deep, showing radiating lines of conidial areas, running out unevenly with central areas thin almost papery but convoluted or wrinkled with ridges; colour bluish-green becoming dull dark green with age; reverse yellow but later shading towards dark almost black areas with the wrinkling of the colony distinctly evident; conidiophores rising from creeping hyphae; penicillus 20.37–37.29  $\mu$  long with walls smooth, mostly monoverticillate, sterigmata 6.78–11.88  $\times$  2.36–3.39  $\mu$  in verticils of 2–8; conidia about 3.4  $\mu$ , globose (Fig. 2).

FIG. 2.—*P. fellutanum*.

a—Monoverticillate penicillus;

b—Biverticillate penicillus. Both  $\times$  1200.4. *P. digitatum* Sacc.

Thom (1930), Thakur &amp; Norris (1928).

Isolated from soil.

5. *P. oxalicum* Currie & Thom.

Thom (1930), Thakur &amp; Norris (1928).

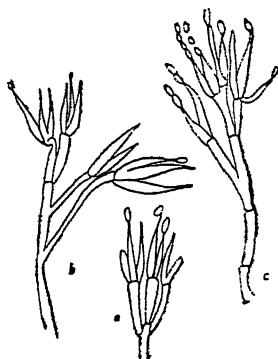
Isolated from soil.

6. *P. atramentosum* Thom.

Thom (1930), Chaudhuri &amp; Umar (1935).

Isolated from Petri-dishes exposed to air.

Colonies upon Czapek's solution agar, velvety, azonate, dull dark green with margin 1-2 mm. wide and a trace of bluish in the newer conidial arrears with reverse in purple brown; conidiophores  $50.92-176.54\ \mu$ ; metulæ  $10.18-12.56\ \mu$ , sterigmata deciduous in mounts,  $10.18-12.56\ \mu$ , closely packed; conidia oval to elliptical,  $3.39-4.1 \times 2.38-3.1\ \mu$ ; penicillus from a verticil of metulæ about  $25\ \mu$  long with the main axis occasionally prolonged to form a second superposed verticil,  $40.7-61.1\ \mu$  in total length (Fig. 3).

FIG. 3.—*P. atramentosum*.

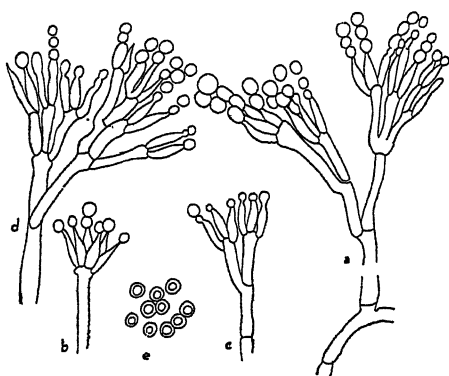
a—Penicillus with metulæ and sterigmata ;  
b-c—Branching conidiophores. All  $\times 540$ .

7. *P. chloro-leucon* Biourge.

Thom (1930), Chaudhuri &amp; Umar (1935).

Isolated from Petri-dishes exposed to air.

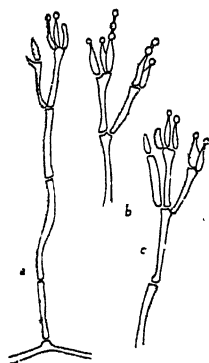
Colonies upon Czapek's solution agar, velvety, with some creeping hyphae about  $1000\ \mu$  deep with white margin 1-1.5 mm. wide, dull deep green to grey green, reverse uncoloured, brownish with age; conidiophores  $2.38-3.4\ \mu$  in diameter: Pencillus a single verticil of sterigmata or a central axis and 1 to 3 branches or metulæ from topmost node, unequal,  $10.18-20.3\ \mu$  long in the same group; sterigmata  $8.48-10.18 \times 2.71\ \mu$  bearing parallel chains of conidia; conidia globose,  $3.73-4.1\ \mu$  (Fig. 4).

FIG. 4.—*P. chloro-leucon*.

a—Formation of conidiophores ;  
 b-d—Penicilli of different forms ;  
 e—Spores. All  $\times 540$ .

8. *P. steckei* Zaleski.

Thom (1930), Chaudhuri & Sachar (1932), Chaudhuri & Umar (1935).  
 Isolated from soil and air (Fig. 5).

FIG. 5.—*P. steckei*.

a—Conidiophore from submerged hypha ;  
 b-c—Penicilli. All  $\times 540$ .

9. *P. cyaneo-fulvum* Biourge.

Thom (1930), Chaudhuri & Umar (1935).

Isolated from exposed Petri-dishes.

Colonies upon Czapek's solution agar, velvety with a broad white zone from 100 to even 500  $\mu$  deep towards the centre where the mass becomes more or less wrinkled, reverse and agar yellow with rich yellow drops ; conidiophores 2.5–3.5  $\mu$  in diameter with smooth walls. Penicillus about 50  $\mu$ , terminal verticil of metulae with irregular branching, branches 20–30  $\mu$

long in pairs or threes; metulæ  $8.84-15.27 \times 2.3-3.1 \mu$  in threes or fours; sterigmata  $6.78-10.18 \times 2.37-3.1 \mu$  in verticils of 2 to 5; conidia globose,  $3.4-4 \mu$ ; a few ovate, caducous (Fig. 6).

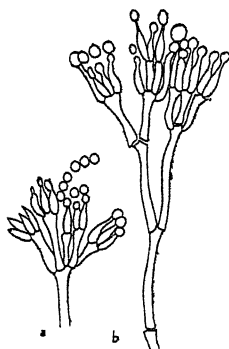


FIG. 6.—*P. cyaneo-fulvum*.

a—Penicillus with metulæ and sterigmata;  
b—Branched conidiophores. All  $\times 540$ .

10. *P. casei* Staub.

Thom (1930), Chaudhuri & Umar (1935).

Isolated from exposed Petri-dishes.

Colonies upon Czapek's solution agar forming thin, tough close-textured felts, buckled and wrinkled, in bluish glaucous to dull green shades,  $150-200 \mu$  deep, reverse yellow to orange brown; conidiophores  $74.7$  to  $227.5 \mu$  long  $\times 3.4-4 \mu$  in diameter, walls granular; penicillus varies from  $44.13-152.17 \mu$  bearing verticils of metulæ  $10.18-16.9 \mu$  (occasionally upto  $23.76 \mu$ ) long,  $1.7-3.1 \mu$  wide; sterigmata  $7.46-10.18 \mu$ , few in the verticil; conidia smooth, globose,  $3.73$  to  $4.4 \mu$  in diameter, produced in long parallel to divergent columns (Fig. 7).

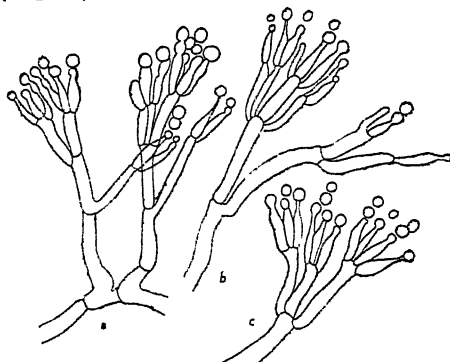


FIG. 7.—*P. casei*.

a—Conidiophore;  
b—Penicillus with rami;  
c—Penicillus with metulæ and sterigmata. All  $\times 540$ .

11. *P. puberulum* Bainier.

Thom (1930), Chaudhuri &amp; Umar (1935).

Isolated from exposed Petri-dishes.

Colonies on Czapek's solution agar, velvety, restrictedly growing, azonate during the rapidly growing period but later dense in central areas, bluish green, later green; reverse tan, agar uncoloured; conidiophores  $67.9-169.7 \times 3.4-4.1 \mu$ , slightly sinuous with walls smooth or more or less roughened; Penicillus  $27.16-135.8 \mu$  consisting of terminal verticil of matulae with branching from a lower node, all elements enlarged and more or less vesicle-like at the apex; sterigmata  $8.4-11.87 \times 2-2.5 \mu$ , 4 to 6 in verticil, conidia smooth, globose,  $3.4-4.75 \mu$  (Fig. 8).

12. *P. terrestre* Jensen.

Thom (1930), Chaudhuri &amp; Sachar (1932).

Isolated from soil (Fig. 9).

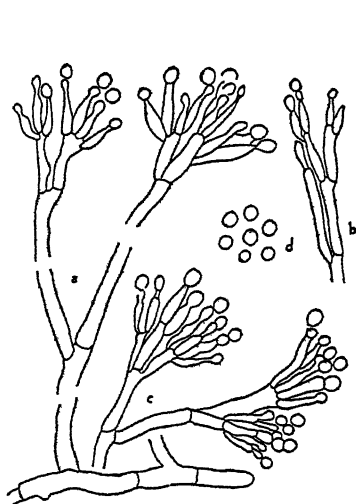


FIG. 8.—*P. puberulum*.  
a—Branched conidiophore arising from submerged hyphae;  
b-c—Penicilli;  
d—Spores. All  $\times 540$ .

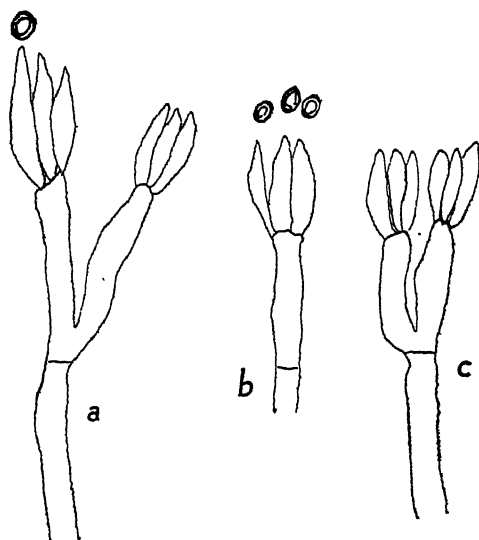


FIG. 9.—*P. terrestre*.  
a-c—Conidiophores with sterigmata and spores.  $\times 1080$ .

13. *P. glaucum* Link, Wehmer.

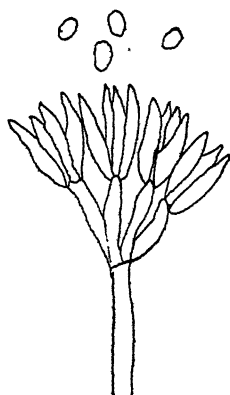
Thom (1930), Thakur &amp; Norris (1928).

Isolated from soil.

14. *P. pinophilum* Hedgcock.

Thom (1930), Chaudhuri &amp; Sachar (1932).

Isolated from soil (Fig. 10).

FIG. 10.—*P. pinophilum*.Conidiophore with metulæ, sterigmata and spores.  $\times 1080$ .

15. *P. tenellum* Cooke.  
Thom (1930), Cooke (1878).  
On rotting leaves of *Symplocos* from Bengal.
16. *P. scopulariopsis brevicaulis* var. *glabra* Thom.  
Thom (1930), Ray & Chaudhuri (1930).  
Isolated from opium in storage, causes losses of morphine in storage.

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# LINKAGE BETWEEN A PANICLE FACTOR AND THE PEARLY-CHALKY MESOCARP FACTOR (Zz) IN SORGHUM.

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There is a wide range of variation in the size and shape of panicles in sorghum. This is due to differences in the length of the rachis, the number of nodes on the rachis, the length of seed branches, the angle of insertion of these branches and the number and distribution of branchlets and spikelets on the side branches. These panicle characters are often a help in separating varieties and races within a species of sorghum. Though there are certain well marked types of panicle, the large number of intermediate types incidental to cultivation under various environmental conditions, makes the pursuit of the inheritance of panicle characters in related varieties particularly difficult. The result has been that there is much of loose nomenclature in the description of this character and in the pursuit of its genetics.

Ramanathan (1924) observes, " There is a huge variation in this character. The stout compact head of Type 1 of *Tella jonna* and the lean lax head of *Irungu* may form the extreme limits. All sorts of intermediates between these two exist. That a number of factors are concerned here is evident from the fact that different types of intermediates breed true. If pure strains happen to cross with another possessing the complimentary allelomorph, compactness behaves as a pure recessive in the simple Mendelian ratio of 3 : 1." Karper (1931) while studying the inheritance of several of the head characters in *kafr* concludes as follows : " Number of nodes in the head, length of rachis, and number of seed branches are three of the quantitative characters which appear to be inherited in a definite Mendelian manner and due to a single factor. The inheritance of these three characters may be governed by the same factor. Length of seed branches also appears to have a single factor involved in its inheritance." Martin (1936), who has gathered together the Mendelian experiences in sorghum from literature or from data " reported to or obtained by him " mentions definitely that in the panicle " short branches " are a simple dominant to " long branches ". In the absence of connected published literature the details of the work leading to this conclusion are not available.

In this paper we record the details of an experience in which there is a linkage between one of the factors for panicle shape and the grain mesocarp factor *Z* (Rangaswami Ayyangar *et al.*, 1934), determining the thickness of mesocarp and the uneven deposition of starch therein. Indications of the occurrence of this have already been given (Rangaswami Ayyangar, 1934, *a, b*).

This experience occurred in the group of sorghum, *Sorghum dochna* (Forsk.) Snowden. In this variety in the year 1928 one of the single plant cultures, A.S. 2388, proved to be a natural hybrid and segregated giving two types of panicles, "Loose conical", and "Compact spindle" (Plate II) in a ratio approximating 3:1. It also segregated for the nature of the grain—pearly and chalky—in a similar ratio. Jointly the two sets of characters gave a 2:1:1 ratio of loose panicle with pearly grain, loose with chalky grain, and compact panicle with pearly grain; the fourth group, compact panicle with chalky grain was absent.

From the above family seven selections were carried forward and an  $F_3$  was raised. The behaviour of this  $F_3$  is given below.

TABLE I. A.S. 2388— $F_3$  Selections.

Family Nos.	Character of Selection	Progeny Behaviour			
		Panicle—		Compact-spindle	
		Grain—			
		Pearly	Chalky	Pearly	Chalky
A.S. 2694	Loose and Pearly	34	18	13	..
A.S. 2697	do.	53	21	23	..
A.S. 2696	do.	63	23	..	..
A.S. 2698	Loose and Chalky	..	Pure	..	..
A.S. 2699	do.	..	Pure	..	..
A.S. 2701	Compact and Pearly	..	..	107	26
A.S. 2700	do.	..	..	Pure	..

From this  $F_3$  generation it was clear that the two sets of factors for panicle and grain were linked to each other though not absolutely. From this  $F_3$  a fourth generation was raised and the behaviour of the selections is given in Table II.

TABLE II. A.S. 2388— $F_4$  Selections.

Family Nos.	Character of Selection	Progeny Behaviour				
		Panicle—  Grain—	Loose-conical		Compact-spindle	
			Pearly	Chalky	Pearly	Chalky
<i>From A.S. 2697 :</i>						
A.S. 2851 ..	Loose and Pearly	86	51	22	..	
A.S. 2853 ..	do.	52	31	18	..	
A.S. 2854 ..	do.	75	47	35	..	
A.S. 2855 ..	do.	74	50	18	..	
A.S. 2856 ..	do.	51	39	35	..	
A.S. 2852 ..	do.	121	..	26	..	
<i>From A.S. 2696 :</i>						
A.S. 2847 ..	Loose and Pearly	78	32	..	..	
A.S. 2849 ..	do.	90	27	..	..	
A.S. 2850 ..	do.	103	21	..	..	
A.S. 2848 ..	do.	Pure	..	..	..	
<i>From A.S. 2701 :</i>						
A.S. 2857 ..	Compact and Chalky	..	..	..	Pure	
A.S. 2858 ..	do.	..	..	..	Pure	

The trend of this experience being clear and all aspects of this character having been met with and the character pair, loose and compact, remaining constant through seasons, artificial crosses were made with the following parents isolated from the  $F_3$  and  $F_4$  generations given above :—

<i>Selection No.</i>	<i>Panicle</i>	<i>Grain</i>
A.S. 2848	Loose conical	Pearly
A.S. 2699	„ „	Chalky
A.S. 2700	Compact spindle	Pearly
A.S. 2858	„ „	Chalky

From these artificial crosses and their selfed  $F_1$ s, an  $F_2$  generation was raised and the behaviour of the progeny is tabulated below ;—

TABLE III.  $F_2$  from Artificial Crosses.

Family Nos.	Progeny Behaviour				
	Panicle—		Loose-conical		Compact-spindle
	Grain—	Pearly	Chalky	Pearly	Chalky
Parents					
A.S. 2699 .. ..			♂		
A.S. 2700 .. ..				♀	
$F_1$ A.S. CLXXXVII .. ..		$F_1$			
A.S. CXCIH .. ..					
$F_2$ A.S. 3918 .. ..		141	66	72	..
A.S. 3919 .. ..		133	61	50	..
A.S. 3925 .. ..		144	70	75	..
TOTAL ..		418	197	197	..
Parents					
A.S. 2848 .. ..		♂			
A.S. 2858 .. ..					♀
$F_1$ A.S. CLXXXVIII .. ..		$F_1$			
$F_2$ A.S. 3920 .. ..		167	3	..	36
A.S. 3921 .. ..		151	1	..	54
A.S. 3922 .. ..		124	2	..	42
TOTAL ..		442	6	..	132
Parents					
A.S. 2699 .. ..			♀		
A.S. 2848 .. ..		♂			
$F_1$ A.S. CXCIH .. ..		$F_1$			
$F_2$ A.S. 3924 .. ..		58	19		
Parents					
A.S. 2700 .. ..				♂	
A.S. 2858 .. ..					♀
$F_1$ A.S. CXC .. ..				$F_1$	
$F_2$ A.S. 3923 .. ..				108	34

It will be seen from the above table that there has been a repetition of the 2 : 1 : 1 ratio in three families in confirmation of the repulsion phase of the linkage. The chief interest in this experience is in the segregation of the three families which show the coupling phase. In these families six cross-overs were obtained in the group "Loose chalky". The frequency of crossing over calculated from this coupling phase is 1.07 per cent.

Based on a cross-over value of 1.07 per cent. the theoretical distribution of the four groups in the  $F_2$  segregation should be as follows :—

TABLE IV.

	Panicle—	Loose-conical		Compact-spindle	
	Grain—	Pearly	Chalky	Pearly	Chalky
Coupling phase—					
Actual (A.S. 3920, A.S. 3921, and A.S. 3922) ..		442	6	..	132
Expected .. ..		432	3	3	142
		$\chi^2 = 6.93$	$.05 > P > .02$		
Repulsion phase—					
Actual (A.S. 3918, A.S. 3919, and A.S. 3925) ..		418	197	197	..
Expected .. ..		406	203	203	.02
		$\chi^2 = .72$	$P > .05$		

This experience of close linkage is very similar to the experience obtained by Imai (1925) in the Japanese Morning Glory. It will thus be seen that there is a very close linkage between a factor for panicle shape and Z, the factor for pearly grains.

It has been previously recorded (Rangaswami Ayyangar, 1933 and Rangaswami Ayyangar *et al.*, 1934) that pearly grains are a simple dominant to chalky grains. Chalky grains have a thick mesocarp with an uneven deposit of starch. These grains absorb water quicker than the pearly grains. The pearly grains are lustrous. Many of the grain sorghums of the hot countries are of the compact headed type and belong to *Sorghum durra* Stapf

and *S. cernuum* Host distributed in India, Arabia, Palestine and Egypt and are predominantly pearly. For a bird's eye view the mass of grains that are exposed to the scorching sun present a pearly and lustrous surface. An examination of the incidence of the nature of the grain in varieties of *S. dochna* shows that there is a preponderance of chalky grains and an abundance of loose panicles. For a bird's eye view less of the grain surface is exposed and more of the shining glume. It would appear that in these interesting experiences from the above crosses, there is a possible explanation of the selective influence that perpetuated a pearly grain in a compact earhead. In any incursion of pearly grains into a predominantly chalky grained and loose headed *S. dochna* population, the linkage repercussions manifest themselves and the odd cross-overs resulting therefrom furnish the other minority groups in the variety. The extent to which such subgroups survive as economic cultivated varieties in their respective tracts varies with their survival value therein.

It has also been recorded (Rangaswami Ayyangar, 1932) that in varieties belonging to the group *S. dochna* the loose conical head shape is a simple dominant to the adpressed, compact spindle panicle. An analysis of earheads of these two types was made and the average values of the various panicle characters are presented below. They are from 35 earheads for each type and are from three seasons.

*Summary of Panicle Measurements.*

Character	Loose-conical	Compact-spindle
Length of rachis .. ..	25.8 cm.	23.0 cm.
No. of whorls .. ..	10.5	10.8
No. of primary branches per whorl ..	7.1	7.4
Average length of primary branches ..	10.9 cm.	6.1 cm.
No. of secondary branches per primary branch .. ..	7.7	5.9
No. of sessile spikelets per earhead	3234	2228

From the above statement it will be noticed that though in the first character, *viz.*, length of rachis, there is a slight pull down in length in the compact-spindle panicle, it may be generally stated that in the first three

characters the two types do not materially differ. The real difference is in the length of the primary branches. This affects the number of secondary branches and also the number of sessile spikelets on them. Both the earheads are in reality conical owing to the decreasing length in the panicle branches from the base to the tip. In the loose-conical earhead the side branches stream out and preserve the conical shape. In the compact-spindle, though, if all the branches are spread out artificially, the appearance will be a sharp cone, the earhead looks spindle-shaped for the reason that there is an absence of the usual cushiony appendage at the base and in the axil of the panicle branches. In the loose earheads the branches make an angle of  $40^{\circ}$  to  $50^{\circ}$  with the rachis while in the compact ones the angle is less than  $5^{\circ}$ . This closing up of the panicle branches has a slight effect on the angle which the leaves make with the stalk. The plants with spindle-shaped earheads have their leaves more erect in comparison with the leaves of plants with loose conical earheads (Pl. II).

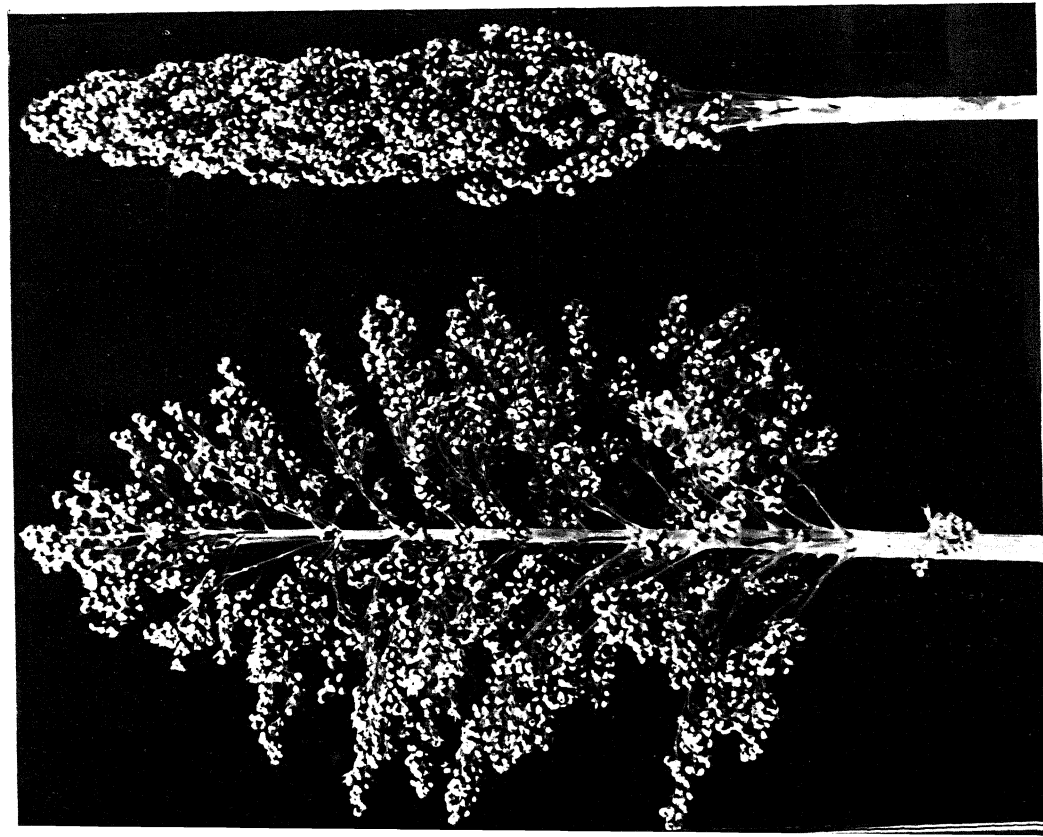
A long panicle branch with the attendant increase in the number of secondary branches thereon, weighs down under the weight of increased grains and gives a loose panicle the characteristic appearance, in which the grain surface is tending to face more sideways and downwards. Whereas in a compact spindle-shaped earhead the mass of grains have to face a scorching sun and in this the lustrous pearly grains are an aid to the plant.

A factor designated  $Pa_1$  is responsible for long panicle branches;  $pa_1$  produces short panicle branches, which, due to the absence of the pulvinus-like appendage at their base results in the branches being adpressed to the central stalk giving the earhead a spindle shape. This factor operates in earheads in which the general trend is for the earhead to be conical in shape. The collateral effects of this gene in a compact spindle earhead are to slightly shorten the length of rachis, lessen the number of secondary branches and reduce the number of sessile spikelets.

The factors  $Pa_1$   $pa_1$  are closely linked to the mesocarp factors  $Zz$  with a cross-over percentage of 1.07.

#### Summary.

In the group of sorghum *S. dochna* (Forsk.) Snowden two types of panicles have been met with. In the loose-conical type the panicle branches are long and the earhead is conical in appearance owing to their decreasing length from base to tip. In the compact-spindle type this conical disposition is also there, the cone being narrower; but the panicle branches are considerably shorter in length and due to the absence of the pulvinus-like structure

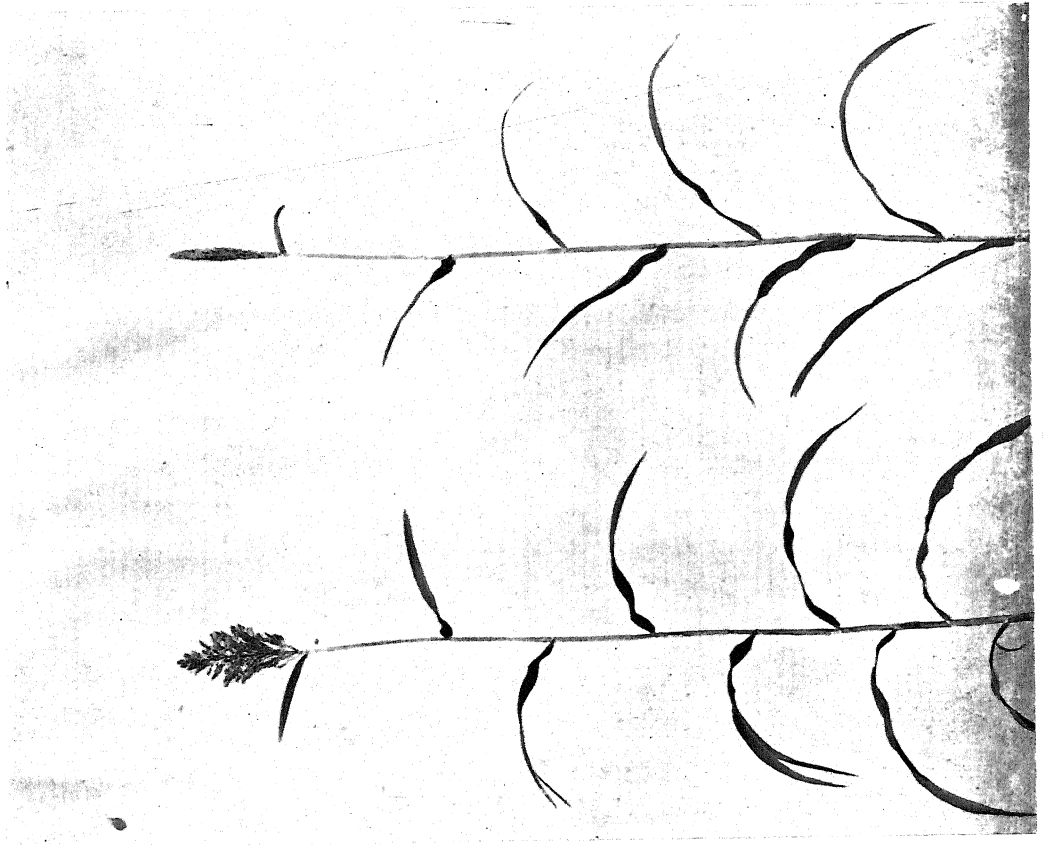


Earheads

Loose  
Conical

Compact  
Spindle

*Sorghum dochna* (Forsk.) Showden.



Slightly  
erect

Leaves

Normal



in the axil of the panicle branches, they are adpressed to the central rachis giving the earhead a spindle-shaped look. In these spindle-shaped earheads, the rachis is slightly reduced in length, the number of side branches decreases and the number of fertile spikelets (grains) also decreases. The leaves also make a slightly narrower angle with the stem.

A factor  $Pa_1$  produces loose conical earheads : factor  $pa_1$  results in compact spindle-shaped earheads. These factors  $Pa_1$  and  $pa_1$  affecting the panicle shape are closely linked to the factors  $Zz$  governing the thickness of mesocarp and the deposit of starch in it, with a cross-over value of 1.07 per cent.

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## Errata.

Vol. VIII, No. 3, September 1938.

- Page 116, line 9—for "*Spirogyra parvala*" read "*Spirogyra parvula*".
- Page 116, line 18—omit "a" before "Chytridiaceous fungi".
- Page 119, under explanation of Fig. 1—for "*F.-Foveolat*" read "*F.-Foveolate*".
- Page 120, line 8—for "spore" read "spores".
- Page 122, line 18—for "Tenda" read "Tanda".
- Page 123, line 14—for "adjoins" read "adjoined".
- Page 126, under explanation of Fig. 8B—read "zygospore" for "zygopsore".
- Page 127, line 5 from below—add "out" after "sticking".
- Page 130, line 7—for "village" read "villages".
- Page 130, line 3 from below—for "purpule" read "purple".
- Page 134, line 9 from below—for "cell" read "cells".
- Page 139, in para marked "Reproduction", line 4—read "Fruiting cells"  
for "Fruiting cell".
- Page 139, line 8 from below—for "zygospore" read "zygospores".
- Page 141, in para 4—for "*Zygnomopsis*" read "*Zygnemopsis*".



# OBSERVATIONS ON SOME ZYGNEMALES FROM NORTHERN INDIA—PART I.

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THE algæ which form the subject-matter of this paper were collected from Lahore, Jullundar and Hoshiarpore districts in the Punjab from 1929 to 1931 and from Saharanpore, Fyzabad, Gonda, Azamgarh, Basti, Bahraich and Barabanki districts in the United Provinces from 1934 to 1938. The richest collection was secured from Fyzabad District which was thoroughly explored from October 1936 to June 1938. Fyzabad District lies in the north-east of Oudh province of the United Provinces, between the parallels  $26^{\circ}9'$  and  $26^{\circ}50'$  north latitude and  $81^{\circ}41'$  and  $83^{\circ}8'$  east longitude. It is a very centrally situated district of Oudh province, and is separated from Gonda and Basti districts in the north by Sarju river, to the south and south-west lies Sultanpur District, to the west is Barabanki, and to the east is Azamgarh.

In general aspect Fyzabad District consists of a level plain of a generally uniform character, the only variation in general flatness being caused by numerous streams, drainage channels, and jhils (small lakes). The average elevation of the country above the sea-level is about 300 feet.

The members of the order Zygnemales though found all over Northern India in fresh-water ponds, puddles, paddy fields and jhils, are most abundant in slowly flowing fresh-water streams called nadis, which usually empty themselves in big rivers. In Fyzabad District there are three nadis, Tons, Pikia and Thirua all of which are tributaries of Sarju, and are very interesting from the algal point of view. Of these Tons yielded a very rich collection of Zygnemales. Its two main tributaries are Marha and Bisui nadis, which join each other 5 miles west of Akbarpur to form Tons. The course of this stream is exceedingly tortuous. It is frequently dammed in the months of March and April for purposes of irrigation and catching fish, and an outlet is usually maintained on one side of the dam due to which a slight flow of water is maintained. Members of Zygnemales begin to appear in the early part of December, become dominant in the months of February and March, and begin to disappear in the last week of April, very few forms lingering on

to May. Similar is the case with Thirua which joins Sarju river near Tanda town, and Pikia which empties itself in the same river in the eastern corner of this district. The Sarju river itself is useless from the algal point of view. From the month of June to October its water remains very turbid, the river attains an immense size and volume, and the velocity of the current is too swift for the growth of any algæ. Only during the months of January and February, when it very much shrinks in size, that some sterile species of *Spirogyra* were collected.

#### LAKES AND PONDS.

The drainage is defective in most parts of the district, and in depressed parts jhils or small lakes are formed, some of which retain water throughout the year. Collections were made from Darwan and Ram Nagar jhils in Tehsil Akbarpur, and Bukia and Sardhana jhils in Tanda. There are a number of small ponds scattered in nearly all the villages of the district, used for irrigating crops, which were also explored. These ponds periodically dry up, and most of these are perfectly dry by the end of April.

#### GEOLOGY, SOIL AND RAINFALL.

We find only Gangetic alluvium in most of the districts in the United Provinces from where collections were made. The only mineral found is "Kankar", a kind of limestone occurring in the form of nodules and blocks, nearly everywhere, and very commonly in Usar land. The average rainfall of Fyzabad District is 42 inches annually, and most of this falls in the months of July, August and September.

#### Periodicity in Occurrence and Reproduction.

The members of the order Zygnemales show a marked periodicity in occurrence and reproduction in Northern India, which is closely connected with temperature, rainfall and hydrogen-ion-concentration of water. The normal life of a member of Zygnemales in Northern India is divisible into five periods as follows:—

1. *Period of Spore Germination.*—All spores do not germinate as soon as water is available. Though all our ponds and nadis are full of water from the month of July onwards, very few species appear even as late as September. There are some species like *Sirogonium ventersicum*, *Spirogyra dædalea* and *S. Oudhensis* in which spores germinate in the end of August, the alga becoming abundant in the end of September, while in majority of forms the spores germinate in December, there being a long period of dormancy.

2. *Period of Growth and Vegetative Development.*—Forms which germinate in late August show a period of vegetative development for about a

month or so when active growth takes place with extensive cell division and storage of food material. The forms which become dominant in January show extensive vegetative development in December and early January. This period of growth and vegetative development is very short in North India.

3. *Period of Conjugation or Aplanospore Formation.*—As soon as filaments become sufficiently numerous conjugation or aplanospore formation starts. In early Autumn forms conjugation starts in September or early October, while in late Winter and Spring forms conjugation starts in December and January.

4. *Period of Spore Ripening.*—In early Autumn forms spores begin to ripen in October and by early November all spores are ripe, which in late Winter and Spring forms spores ripen in March and early April.

5. *Period of Dormancy.*—The spores of all Zygnemales in Northern India lie dormant during the hot and dry months of May and June when nearly all ponds and nadis dry up. The late Winter and Spring forms have a longer period of dormancy as compared with late Autumn and early Winter annuals. While the spores of the latter germinate in September those of the former lie dormant from the end of April to early December. It seems surprising that in spite of their immersion in water from the middle of July to the end of November, the spores of these forms germinate so late.

#### THREE GROUPS OF ZYGNEMALES IN NORTHERN INDIA.

Members of the order Zygnemales form the dominant vegetation in our fresh-water nadis, jhils and ponds. With reference to their time of germination, vegetative development, conjugation and period of spore-ripening, we may divide the Northern Indian Zygnemales into three groups as follows:—

##### I. *Late Autumn and Early Winter Annuals.*

The species which are included in this group usually germinate in the end of August or in the middle of September after the close of monsoon rains, become abundant by the last week of September or middle of October when they start conjugating, and produce ripe zygospores in October and November. Some of these forms may linger on to December. So far the present author's observations go, this group includes about 2 species of *Mougeotia*, 1 of *Debarya*, 3 of *Zygnema*, about 7 of *Spirogyra* and 1 of *Sirogonium*. It is a noticeable fact that most of the species of *Spirogyra* included in this group have relatively broad filaments.

I. *Late Autumn and Early Winter Annuals.*

No.	Genus	No.	Species.
I	<i>Debarya.</i>	1	<i>D. costata</i> sp. nov.
II	<i>Mougeotia.</i>	1	<i>M. tenuis</i> Cleve.
		2	<i>M. floridana</i> Trans.
III	<i>Zygnema.</i>	1	<i>Z. cyanosporum</i> Cleve.
		2	<i>Z. collinsianum</i> Transeau.
		3	<i>Z. mucigena</i> sp. nov.
IV	<i>Spirogyra.</i>	1	<i>S. fluviatilis</i> Hilse.
		2	<i>S. nitida</i> (Dillw.) Link.
		3	<i>S. rhizoides</i> sp. nov.
		4	<i>S. crassa</i> Kutz.
		5	<i>S. neglecta</i> (Hass) Kutz.
		6	<i>S. quadrata</i> (Hass) Kutz.
		7	<i>S. jacense</i> sp. nov.
V	<i>Sirogonium.</i>	1	<i>S. ventersicum.</i> Transeau var. <i>melanosporum</i> var. nov.

II. *Late Winter and Spring Annuals.*

In this group those species are included whose spores germinate in late November or early December, show vegetative activity in December when they are green in colour, conjugate in January and early February when they become palish yellow, and produce ripe spores in the end of February and March, or in some cases in April when they become dark-blue or purplish in colour. In the end of April and beginning of May most of the nadis, ponds and small jhils dry up and these remain dry upto the middle of July when monsoon rains start. During this period of drought these Zygnemales perennate in the form of zygospores and aplanospores. Following algae are provisionally described in this group. On further observations some of these might prove to be ephemerals which conjugate twice or thrice in the Winter and Spring months.

II. *Late Winter and Spring Annuals.*

No.	Genus	No.	Species.
I	<i>Mougeotia.</i>	1	<i>M. viridis</i> (Kutz) Witt.
		2	<i>M. gotlandica</i> (Cleve) Witt. var. <i>crassa.</i> var. nov.
		3	<i>M. parvula</i> Hass.
		4	<i>M. quadrata</i> sp. nov.
		5	<i>M. bicalyptata</i> Witt.
		6	<i>M. scalaris</i> Hassal.

No.	Genus	No.	Species.
II	<i>Zygnemopsis</i> .	1	<i>Z. splendens</i> Randhawa.
		2	<i>Z. lamellata</i> „
		3	<i>Z. Indica</i> „
		4	<i>Z. Iyengari</i> „
		5	<i>Z. lamellata</i> var. <i>globosum</i> .
		6	<i>Z. minutum</i>
		7	<i>Z. gracilis</i> sp. nov.
		8	<i>Z. Transeauna</i> sp. nov.
		9	<i>Z. sphærospora</i> sp. nov.
III	<i>Zygnema</i> .	1	<i>Z. Czurdæ</i> Randhawa
		2	<i>Z. cæruleum</i> Czurda.
		3	<i>Z. chalybdospermum</i> Hansg.
		4	<i>Z. giganteum</i> Randhawa.
		5	<i>Z. Oudhensis</i> sp. nov.
		6	<i>Z. Heydrichii</i> Schmidle var. <i>indicum</i> var. nov.
		7	<i>Z. inconspicuum</i> „
		8	<i>Z. sp.</i>
IV	<i>Spirogyra</i> .	1	<i>S. parvula</i> (Trans.) Czurda.
		2	<i>S. flavescens</i> (Hass) Kutz.
		3	<i>S. Hassallii</i> (Jenn) Petit.
		4	<i>S. dubia</i> Kutz.
		5	<i>S. Grevilliana</i> (Hass) Czurda.
		6	<i>S. Sahnii</i> sp. nov.
		7	<i>S. affinis</i> (Hass) Kutz.
		8	<i>S. Jurgensii</i> (Kutz.)
		9	<i>S. bellis</i> Cleve.
		10	<i>S. condensata</i> (Vauch) Czurda.
		11	<i>S. Gætzæ</i> Schmid.
		12	<i>S. paludosa</i> Czurda.
		13	<i>S. foveolata</i> (Trans.) Czurda.
		14	<i>S. Tandæ</i> sp. nov.
		15	<i>S. Manoramæ</i> sp. nov.
		16	<i>S. aplanosporum</i> „
		17	<i>S. Oudhensis</i> „
		18	<i>S. Skujæ</i> „
		19	<i>S. unduliseptum</i> „
		20	<i>S. setiformis</i> (Roth.) Kutz.

No.	Genus	No.	Species
		21	<i>S. submaxima</i> Transeau var. <i>inflata</i> var. nov.
		22	<i>S. lambertiana</i> Transeau.
		23	<i>S. gallica</i> Petit. var. <i>bichromato-</i> <i>phora</i> var. nov.
V	<i>Sirogonium</i> .	1	<i>S. sticticum</i> Kutz.

### III. Ephemerals.

Under this term Transeau described forms like *Botrydium* which have vegetative cycles of a few weeks duration. This term aptly describes two species of *Mougeotia* and *Spirogyra* which came under the present author's observations from October 1936 to May 1938 in Fyzabad District. These are as follows :—

No.	Genus	No.	Species
I	<i>Mougeotia</i> .	1	<i>M. sphærocarpa</i> Wolle.
II	<i>Spirogyra</i> .	1	<i>S. dædalea</i> . Lag.

Of these algæ, *M. sphærocarpa* was found conjugating, producing ripe zygosporos in all months from September to March. Within a month or so this alga completes its life-cycle, its spores germinate, filaments grow quickly, conjugation takes place, and spores ripen. *Spirogyra dædalea* was also seen with ripe spores in all months from December to March.

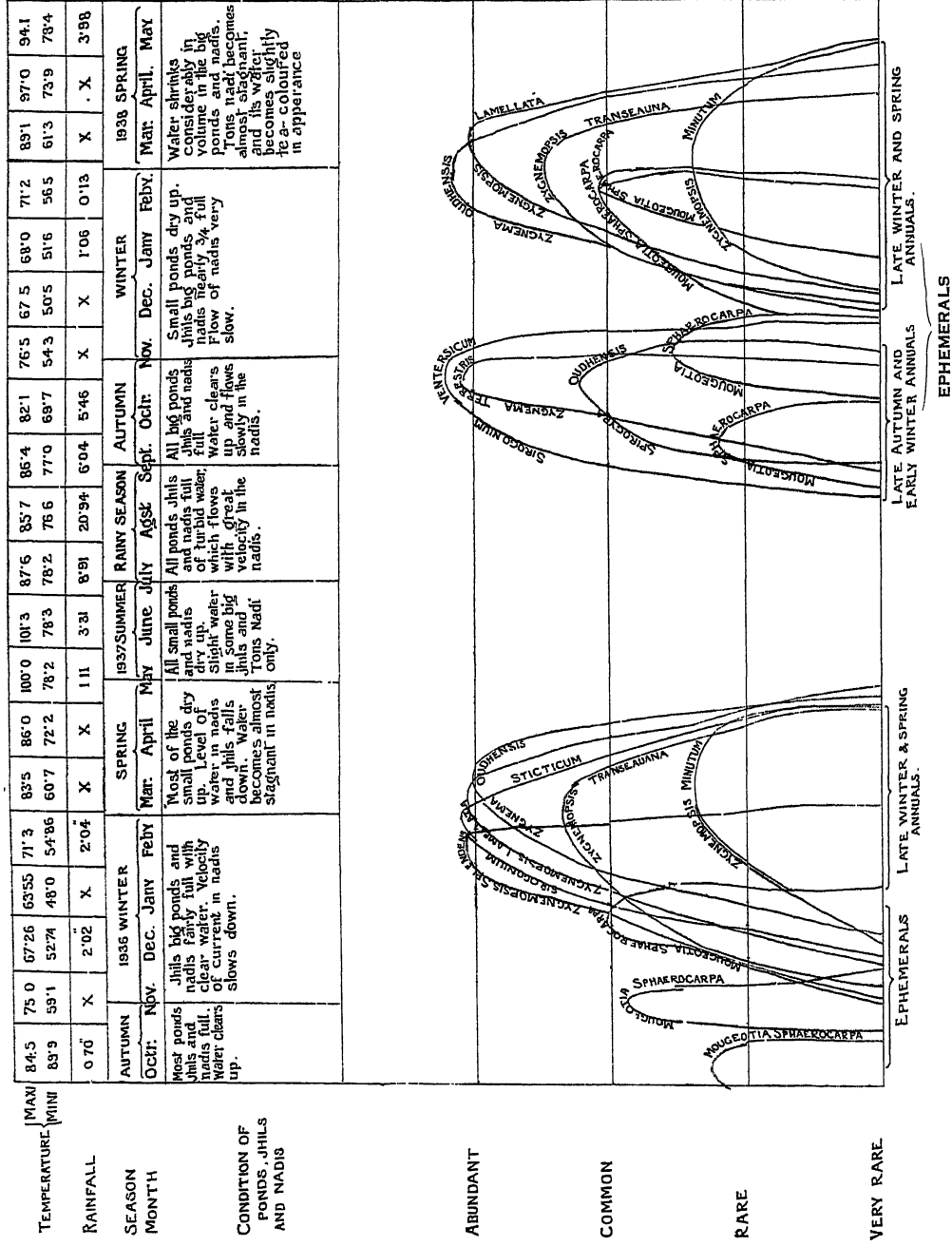
### Succession in Zygnemales in Fyzabad District.

Succession of algæ in Tons nadi near Akbarpur, in a tributary of Thirua nadi near V. Mamrezpur in Tanda Tehsil, and in a pond near V. Mhow Shiwala was observed from October 1936 to May 1938. Collections of algæ were made twice a month in most cases, and once a month in every case during the algal season from September to May, and during the dry period notes were made about the condition of water in all months. Thus it was possible to trace the life-history of 4 species of *Zygnemopsis*, 2 of *Zygnema*, 1 of *Mougeotia* and 2 of *Spirogyra*.

Tons nadi keeps water throughout the year. Its water remains turbid from July to middle of October, and flows with great velocity. In the end of October when the monsoons completely stop, its water clears up and the stream ebbs down leaving numerous puddles at its sides in which sterile filaments of a broad species of *Spirogyra* were observed. At the same time the velocity of the current also decreases, and the banks of the nadi become covered with *Polygonum glabrum*. In the middle of November *Nostoc* sp.,



Chart showing Relative Frequency, Time of Conjugation and Spore-Ripening of some species of  
*Zygnemopsis*, *Zygnema*, *Mougeotia* and *Spirogyra* in Fyzabad District.  
 From October 1936 to May 1938.



*Glæotrichia* sp., *Oscillatoria* sp., *Oedogonium* sp. as well as *Cladophora glomerata* on shells of a gastropod, were observed. It is only in early December that the Zygnemales appear in the main stream. Sterile filaments of *Zygnema Oudhensis*, *Zygnemopsis lamellata*, *Zygnemopsis Transeauana* and *Sirogonium sticticum* are seen. *Zygnemopsis minutum* makes its appearance in the end of December. In January these algæ become quite common and their vegetative filaments fill the main stream. At the same time the phanerogamic vegetation on the sides of the nadi also increases, and *Typha latifolia*, *Sagittaria sagittifolia*, *Ranunculus sceleratus* and *Ammania baccifera* cover the banks of the nadi, while *Vallisneria spiralis*, *Naias* sp., *Limnanthemum* sp. and *Hydrilla verticillata* appear in the main stream. In January and early February all the above-mentioned species of *Zygnemopsis*, *Zygnema* and *Sirogonium* conjugate, and become so abundant that they fill the main stream completely. In March all these species produce ripe spores, which linger on to April. By the middle of May, all Zygnemales disappear, and water also shrinks in volume very much.

In the tributary of Thirua nadi near V. Mamrepore, the pioneers after rains in September are also Myxophyceæ. In the fields around the stream *Zygnema terrestris* appears in green felt-like masses, and becomes quite common in early September when it starts conjugation. In the end of October this alga produces ripe zygospores and it disappears by the middle of November. A parallel course of events in the stream itself is shown by *Mougeotia sphærocarpa*, which however produces two more generations by the end of February, when this little stream finally dries up.

In the pond near Mhow Shiwala, the first alga to appear by the end of August was *Sirogonium ventersicum*, which became abundant in September when it started conjugating, produced ripe spores in October and disappeared in the first week of December. In the neighbouring pond of Mhow Jadubanspur *Zygnemopsis splendens* appeared in late November, conjugated in December, produced ripe zygospores in February and disappeared in the first week of March. This pond completely dried up in the end of April.

The relative frequency, time of conjugation and spore-ripening of these algæ are shown in the chart. Thus we see that a regular drama is played in our ponds, jhils and fresh-water streams, which otherwise appear so calm and placid. Members of Zygnemales germinate, vegetate, conjugate, produce ripe spores, disappear from view, and perennate in the form of zygospores and aplanospores in the dried mud of our ponds, jhils and nadis.

### Some Peculiarities in Reproduction.

A number of peculiarities and abnormalities were observed in the process of reproduction in North Indian Zygnemales. In *Zygnema giganteum* it was noticed that while some filaments were showing isogamous conjugation exclusively there were others found in the same material which were conjugating in an anisogamous fashion. In *Zygnema Oudhensis* sp. nov. a similar situation was observed. Transeau's remarkable *Z. Collinsianum* was also collected: in this form the zygospores were found almost anywhere in the same couple of conjugating filaments.

In *Spirogyra parvala* (Trans.) Czurda, a form reproducing mainly by lateral conjugation, a sort of morphological anisogamy was also observed. In this form the female cells were found to be very much swollen, were almost flask-shaped in appearance, and it appears more probable that the male gamete passed into the female cell through the middle part of the septa rather than in the traditional method of passing through one side.

In *Spirogyra Sahnii* sp. nov. a form reproducing by means of lateral conjugation, a very much swollen male cell was seen connected with the female cell by means of a tubular structure. This alga was infested with a Chytridiaceous fungi, and it is possible that such abnormal conjugation canals may be the result of fungal infection. Such tubular conjugation canals have been reported by De Bary in *Zygnema insigne* (Hass) Kutz.

The most remarkable species which came under the author's observation is *Zygnemopsis minutum*. In its vegetative structure this alga looks like a colonial form of *Cylindrocystis*. The cells of the filaments are very loosely connected, and conjugation takes place between dissociated free-floating cells. These free-floating cells may meet and fuse in any position and this explains the great variety in the shape of zygospores, which may be triangular with three horns, or with four horns of varying lengths, and in some cases may be even two-horned sickle-shaped bodies resulting from the end to end conjugation of two free-floating cells. These sickle-shaped spores were at first described as aplanospores, and only after a number of observations it was found that these are abnormal zygospores. *Z. minutum* bridges the gap between Saccoderm desmids and filamentous Zygnemales, much more effectively than even *Zygnemopsis desmidioides* = (*Debarya desmidioides* West).

### Asexual Reproduction.

Asexual reproduction takes place by means of akinetes, azygospores, and aplanospores in some species of Zygnemales.

1. *Akinetes*.—An akinete may be defined as a whole cell which develops thick walls, and accumulates starch and other food materials without very noticeable change in its original shape. In this case secondary thickening of the original wall of the vegetative cell takes place and no secondary walls are laid down. Akinetes were abundantly seen in *Zygnema giganteum*, a species in which whole filaments become converted into chains of brick-shaped orange-coloured akinetes.

2. *Aplanospores*.—These spores develop directly inside vegetative cells by contraction of cell-contents, and development of a secondary wall similar to that of the zygospores. These were found in *Zygnemopsis Transeauna*, a form which exclusively reproduces by means of aplanospores and also in *Z. Iyengari* and *Z. minutum*.

3. *Azygospores or Parthenospores*.—These are usually arrested gametes which instead of fusing, develop thick walls and become spore-like bodies. These were observed in *Spirogyra dædalea*, *S. condensata* and *S. Sahnii*, and also in *Zygnema giganteum*.

#### Characteristics Used for Generic and Specific Identification.

The consideration of the following characteristics is important for generic and specific identification of species in Zygnemales.

1. *Vegetative Cells*.—The length and breadth of vegetative cells should also be measured. The breadth of vegetative cells varies within a very small range, and should be measured at the septa.

2. *Chloroplasts*.—There are three main types of chloroplasts seen in North Indian Zygnemales; the axial plate-like, the stellate and spiral type. The axial plate-like chloroplasts with a number of pyrenoids in one or more rows are seen in *Debarya* and *Mougeotia*, while the stellate type with numerous modifications is seen in *Zygnema* and *Zygnemopsis*, while the spiral type is seen in *Spirogyra* and in a modified form in *Sirogonium*. The number of chloroplasts and their spirals should also be observed in the species of *Spirogyra*.

3. *The Septa*.—The character of the septa or end walls of the cells is important for specific determination. There are two common types of septa seen in the North Indian species, as follows:

(i) *The Plain Type*.—This is by far the commonest type of septum seen in Zygnemales. In this case also the end walls may be straight touching each other or swollen.

(ii) *The Replicate Type*.—In this type the middle lamella develops a ring-like ingrowth on either side. Two varieties of this type may be seen in different species of *Spirogyra*.

(a) *Typically Replicate*.—In this case a cylindrically ring-like growth is seen.

(b) *Semi Replicate*.—In this case only a half-ring like growth on either side of the septa is seen and the middle lamella looks like the diagonal of a parallelogram.

4. *Conjugation Canals*.—In species of *Spirogyra* it should be observed whether the conjugation canals are formed by the male cell alone or by both cells.

5. *The Shape of Fruiting Cells*.—The shape of the fruiting cells containing the zygospores is important from the point of view of specific identification. The following are the main shapes of fruiting cells seen in *Zygnemales* :

(i) *Cylindrical Type*.—In this case the female cell is cylindrical in shape and the zygospore almost touches its sides without compressing it.

(ii) *Inflated Type*.—In this case the walls of the female cell bulge out. This may be divided into two sub-types :

(A) *Compressed inflated*.—In this case the zygospore is too big for the space available in the conjugation canal, and it presses against its walls making it bulge out.

(B) *Loose inflated*.—In this case there is plenty of space available inside the female cell in which the zygospore lies loose. There are following shapes seen in this sub-type :

(1) Inflated on both sides.

(2) Inner wall inflated, the outer straight.

(3) Outer wall inflated, inner straight.

(4) Cylindrically inflated.

6. *The Zygospores*.—The form of zygospores, their pigmentation and sculpturing and the comparative thickness of the various layers of the spore-wall is important for the purposes of specific identification.

(A) *The Shape of the Zygospores*.—There are four primary types of shapes seen in the zygospores, and the other shapes are merely modifications or combinations of these.

(i) *Globose or the Rounded Type*.—This shape is commonly found in the zygospores of *Mougeotia* and *Zygnema*.

(ii) *Ellipsoid*.—This shape is commonly seen in the various species of *Spirogyra*.

(iii) *Obovoid*.—This is also seen in some species of *Spirogyra*.

(iv) *Quadrangular*.—This is a common shape in some species of *Mougeotia*, and has numerous modifications, due to angles of the corners, and concave or convex nature of the sides.

(B) *The Spore Wall*.—In most species of Zygnemales, the zygospore wall is composed of three primary layers, the exospore the outer layer, the mesospore the middle layer, and the endospore the innermost layer. The single or double-layered spores showing the chloroplasts are immature and not suitable for the purpose of identification. It is very unsafe to establish new species in such cases merely because the dimensions or the shape of zygospores are different from that of a known species. Recently Misra<sup>15</sup> described some new species of *Spirogyra* and *Zygnema* from Kashmir which show such immature zygospores with undeveloped sculpturing. Of these it is extremely doubtful if *Zygnema indica*, *Zygnema Kashmiriensis* and *Zygnema sphaerica* are really new species. In some species the exospore and the mesospore are further subdivided into two layers each. The exospore is usually thin and hyaline, and so is the endospore which may be totally suppressed in some species, resulting in a two-layered spore-wall. Beside these three primary thickening layers which are laid down around the zygospores, the remains of the gametangia may also be seen forming the outermost covering in *Zygnomopsis* and *Debarya*.

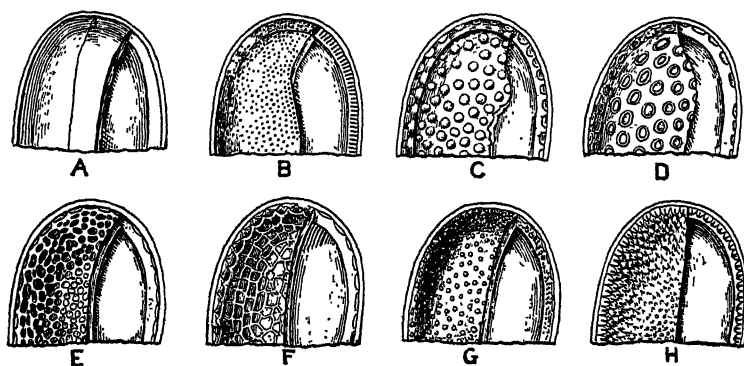


FIG. 1. Various types of sculpturing of the Mesospore.

A.—Smooth ; B.—Punctate ; C.—Scrobiculate ; D.—Pitted ; E.—Reticulate ; F.—Foveolate ; G.—Granulate ; and H.—Denticulate.

Adopted from Jao—All  
Sketches are diagrammatic.

For the purposes of identification the mesospore is the most important. In ripe spores the mesospore is usually variously pigmented, the prevailing

colours being golden yellow, fire-red, chocolate brown, blue and bluish green. The mesospore may be smooth or sculptured, and its ornamentation should be carefully determined. In most cases the sculpturing is quite clear, but in some deeply pigmented forms, it becomes visible only when the material is crushed or mounted in 30 per cent. chromic acid, which dissolves the cell contents. Following are the principal types of markings seen in the Indian species :

1. *Smooth*.—This is quite a common feature of the spore of most of the Indian species of *Spirogyra*, specially the bigger ones (Fig. A).
2. *Punctate*.—In this type small dots are seen (Fig. B).
3. *Scrobiculate*.—In this case small circular pits are seen (Fig. C).
4. *Pitted*.—As compared with the scrobiculate type the pits are more well defined in this type, though these are more shallow.
5. *Reticulate*.—In this type too the spore-wall bears numerous irregular pits which are however so close together that their ridges give the appearance of an irregular network (Fig. E).
6. *Foveolate*.—This differs from the reticulate type in two features, firstly the meshes of the network are wider, and secondly the island in the network represents raised parts and not depressed parts (Fig. F).
7. *Granulate*.—In this case the spore-wall has granular projections on it (Fig. G).
8. *Denticulate*.—The spore-wall is raised into a number of sharp spines in this type (Fig. H).

In some species the mesospore possesses a suture, which may be straight or wavy.

### Systematic Enumeration of the Species Observed.

*Debarya* Wittrock (1872).

Filamentous habit, each cell with a single axile plate-like chloroplast bearing several pyrenoids in a single row. Conjugation isogamous, scalariform, with zygospores in the conjugation canal. No cytoplasmic residue in the gametangia, which become filled with a pectic cellulose colloid. Zygospores not separated by any walls from the gametangia.

*Debarya costata* sp. nov.

(Fig. 2.)

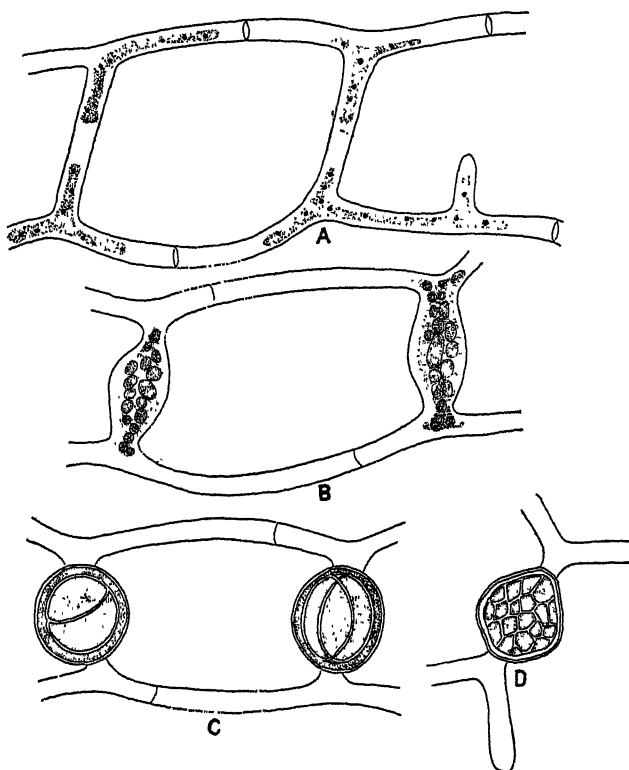


FIG. 2. *Debarya costata* sp. nov.

A.—Shows an early stage in conjugation.

B.—A later stage in conjugation showing the fusion of gametes.

C.—Shows two zygospores with ridges on their surface.

All  $\times 310$ .

D.—Shows reticulations on the surface of a zygospore.

Vegetative cells are 6–10  $\mu$  broad, and each cell has a single plate-shaped chloroplast bearing 4–9 pyrenoids.

*Conjugation*.—Only scalariform conjugation is seen in this species. The conjugation processes are given out in the shape of long cylindrical phallic bodies, which meet and produce long conjugation canals (Fig. A). Conjugation between 3 or more filaments is exceedingly common giving it the appearance of a gauze with rectangular or sub-rectangular meshes. Marked geniculation of the cells takes place during the process of conjugation. After the fusion of gametes, the middle part of the conjugation canal swells up, and the contents show a very much vacuolated appearance. The conjugating filaments become glistening white and solid in appearance, and septa of

the cells become indistinguishable (Fig B) due to secretion of mucilage by the protoplasm in a homogeneous mass.

Zygospores are rounded or elliptical in appearance and are  $22-36\ \mu$  broad, and in elliptical forms may be  $32-42\ \mu$  long. Spore-wall is composed of two layers only; a thick and transparent exospore and a greenish mesospore produced into 1 to 4 wavy ridges (Fig. C). The surface of the zygospores, in between the ridges shows reticulations (Fig. D). Fully ripe spores were not seen.

*Affinities.*—Only six species have been described by Transeau as being included in genus *Debarya* after transference of others to *Zygnemopsis*. Out of these this alga resembles *Debarya formosa* Transeau in the size of its vegetative cells, presence of many pyrenoids on the chloroplasts, and the size of its zygospores. However, it differs from that form in the shape of zygospores, and the presence of more than three ribs and reticulations on the surface of the zygospores. These differences are sufficient to warrant the establishment of a new species which is named as *Debarya costata* sp. nov.

*Habit.*—Found free-floating mixed with *Spirogyra dædalea* in Bukia jhil near Baskhari, tahsil Tenda, district Fyzabad, U.p., during the first week of December 1936.

*Mougeotia* Agardh (1824).

*Filamentous Habit.*—Each cell with an axile plate-like chloroplast containing pyrenoids in one or more rows. Reproduction by means of zygospores and aplanospores. Arms of the gametangia contain cytoplasmic residue varying in colour from pale yellow to dark brown. Conjugation scalariform rarely lateral, usually isogamous, rarely anisogamous. Zygospores become cut off from the gametangia by 2 to 4 walls.

1. *Mougeotia parvula*, Hassal.

*Op. cit.*, Czurda, *Susswasserflora Mitteleuropas*, Heft. 9; and  
Transeau, *The Genus Mougeotia*, page 316.

(Fig. 3.)

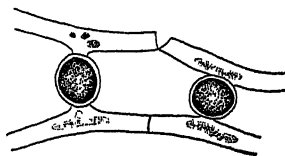


FIG. 3. *Mougeotia parvula* Hass.

× 310.

Shows two ripe zygospores.

Vegetative cells  $6-9\ \mu$  broad, 18–24 times as long. Chloroplast plate-shaped, with 3–6 pyrenoids in a single row.

**Reproduction.**—Conjugation scalariform. Gametangia slightly geniculate. Sporangia are adjoined by two cells. The zygospores are rounded in shape and completely fill the conjugation canal. The mesospore is smooth, thick, and brown in colour. Zygospores are  $16-23\ \mu$  in diameter (Fig. 3).

**Distribution.**—This species has been reported from Germany, Bohemia, Austria, Switzerland, France, Belgium, North America, Brazil, Japan and China.

**Habit.**—Found free-floating in a fresh-water lake at V. Rampur, tehsil Akbarpur, district Fyzabad, U.P., in the second week of January 1937.

2. *Mougeotia bicalyptata* Wittrock (1886).

*Op. cit.*, Czurda, *Susswasserflora Mitteleuropas*, Heft 9, page 64.

(Fig. 4.)

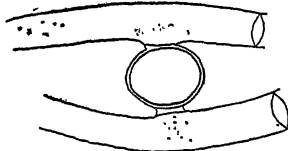


FIG. 4. *Mougeotia bicalyptata*. Witt. × 310.

Vegetative cells  $10-12\ \mu$  broad,  $60-140\ \mu$  long, each with 4-7 pyrenoids.

Sporangia adjoins by two cells. Zygospores globose or slightly depressed with two caps of mucilage, one on each side,  $16-24\ \mu$  in diameter. Mesospore thick, smooth and chocolate brown in colour (Fig. 4).

This form differs from the type in the less extensive nature of the caps of mucilage.

**Habit.**—Found free-floating in a small fresh-water nadi near Mubarakpur, district Fyzabad, on 12th March 1938.

3. *Mougeotia scalaris* Hassal (1842).

Czurda, *Susswasserflora Mitteleuropas*, Heft. 9, page 67; and Transeau, *The Genus Mougeotia*, *Ohio. Jour. So.*, Vol. XXVI, No. 6, p. 317.

(Fig. 5.)

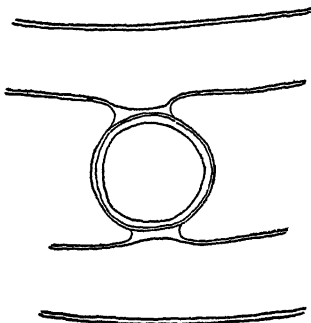


FIG. 5. *Mougeotia scalaris* Hass. × 440.

Vegetative cells  $20-30\ \mu$  broad and  $70-120\ \mu$  long, each with a plate-shaped chloroplast bearing a single row of 5-8 pyrenoids.

Zygospores are globose to ovoid in shape  $26-34\ \mu$  in diameter, brown in colour, median spore-wall smooth brownish yellow. Sporangia adjoined by two cells, spores in the middle of conjugation canals.

*Habit.*—Found in vegetative condition in early February and with ripe spores on 28th March 1938 in a fresh-water stream near V. Mubarakpur, ehsil Tanda, district Fyzabad, U.P.

4. *Mougeotia sphærocarpa* Wolle (1887).

*Op. cit.* Czurda, *Susswasserflora Mitteleuropas*, Heft 9; and Transeau, *The Genus Mougeotia*, p. 319.

(Fig. 6.)

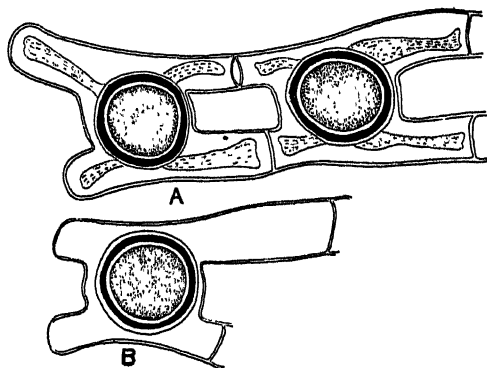


FIG. 6. *Mougeotia sphærocarpa* Wolle.

A.—Shows two semi-mature zygospores.

B.—Shows a fully mature zygospore.

The thick black line shows the mesospore.

× 310.

Vegetative cells are  $20-22\ \mu$  broad and 7-11 times as long. Each cell has a single plate-shaped chloroplast with 6-15 conspicuous pyrenoids in a single row.

*Conjugation.*—Conjugation scalariform. The conjugation canals are immensely wide, in some cases being as wide as  $56\ \mu$ . Due to tremendous width of the conjugation canals distinct geniculation is noticeable in the conjugating filaments. Conjugation between three or more filaments is also not rare.

The zygospores are typically rounded in appearance, and no ellipsoidal specimens were seen. Yellowish remains of the unused part of the protoplasm may be seen in the arms of the gametangia in the case of semi-ripe

specimens (Fig. A), but in fully ripe zygospores the arms get filled with a brownish matter (Fig. B). The zygospore wall is composed of three layers, a light brown exospore, a dark chocolate brown thick and smooth mesospore, and a thin endospore. The zygospores are chocolate brown in colour, and are  $42-50\ \mu$  in diameter. The zygospores get disjoined and float about with the arms of the gametangia sticking out on sides. No aplanaspores were seen.

*Habit.*—Found free-floating in a fresh-water stream near Makrahi, district Fyzabad, United Provinces, in the second week of November 1936. Also collected from various ponds and jhils in district Fyzabad in October, November, December, January and February 1937.

5. *Mougeotia gotlandica* (Cleve) Wittrock, var. *crassa*. var. nov.

(Fig. 7.)

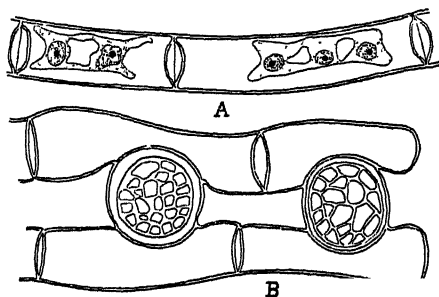


FIG. 7. *Mougeotia gotlandica* (Cleve) Witt.

A.—Shows two vegetative cells with ladder-like chloroplasts.

B.—Shows zygospores.

× 310.

Vegetative cells  $22-24\ \mu$  broad and 3–4 times as long. Each cell contains a single plate-shaped chloroplast with 3–5 conspicuous pyrenoids. In some cases the chloroplasts are fenestrated in the middle, presenting a ladder-like appearance (Fig. A).

*Reproduction.*—Conjugation scalariform with the zygospores in the conjugation canal. Zygospores are cut off from the gametangia by two curved walls. Marked geniculation of conjugating filaments takes place. Zygospores globose,  $36-40\ \mu$  in diameter. Zygospore-wall composed of two layers, a thin, smooth and hyaline exospore, and a thick mesospore (Fig. B). Mature spores were not seen.

This species resembles *M. gotlandica* (Cleve) Witt. in the size of its vegetative cells, but differs from it in having a single row of pyrenoids, and in the larger size of zygospores.

*Habit.*—Found mixed with *Zygnema Oudhensis* in Tons nadi near tehsil buildings, Akbarpur, district Fyzabad, U.P., on 20th February 1937.

6. *Mougeotia viridis* (Kutzing) Wittrock (1872).

*Op. cit.*, Czurda, *Susswasserflora Mitteleuropas*, Heft 9; and Transeau, *The Genus Mougeotia*, p. 323.

(Fig. 8.)

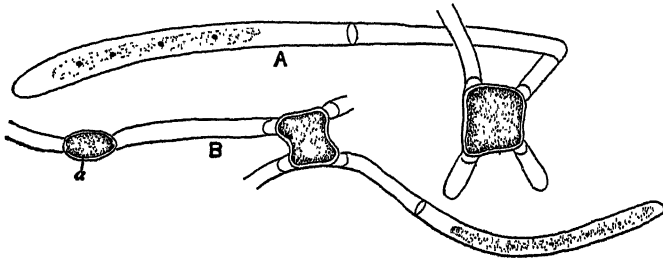


FIG. 8. *Mougeotia viridis* (Kutz) Witt.

A.—Shows a vegetative cell and a ripe zygospore.

B.—Shows an aplanospore and a zygospore.

Both  $\times 310$ .

Vegetative cells  $6-8\mu$  broad, chloroplast plate-shaped with 4 pyrenoids in each.

*Reproduction.*—Conjugation scalariform. Zygospores adjoined by 4 cells squarish with concave sides and retuse angles, and may be seen free-floating with the four horn-like remains of the gametangia attached to them at the corners. Mesospore clear and smooth. Zygospores darkish in colour,  $22-26\mu$  broad and  $22-26\mu$  long (Fig. A). Aplanospores which are oblique-ellipsoid in shape may also be seen in some filaments (Fig. B).

*Habit.*—Found in a fresh-water spring at Tahli Sahib, district Hosharpore, in February 1931.

*Distribution.*—This alga is almost cosmopolitan in distribution and has been reported from England, Germany, Austria, Czechoslovakia, France, Russia, Roumania and North America.

7. *Mougeotia quadrata* sp. nov.

(Fig. 9.)

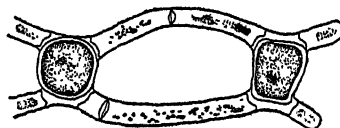


FIG. 9. *Mougeotia quadrata* sp. nov.

$\times 310$ .

Vegetative cells are  $10\text{--}12\ \mu$  broad,  $80\text{--}120\ \mu$  long, each with a plate-like chloroplast bearing 3–6 pyrenoids.

*Reproduction*.—Conjugation is scalariform, with the zygospores extending into both the gametangia. Zygospores are quadrate to globose-quadrate  $22\text{--}23\ \mu$  broad and  $27\text{--}33\ \mu$  long, with an average size of  $27 \times 27\ \mu$ , are yellowish in colour, and have pads of white mucilage at the angles. Spore-wall is smooth (Fig. 9).

*Affinities*.—This form resembles *M. Regalii* Skuja in the size of cells and zygospores, but differs from it in the shape of its zygospores.

*Habit*.—Found free-floating in a fresh-water stream near Mubarakpur, district Fyzabad, on 10th February 1938.

8. *Mougeotia tenuis* (Cleve) Wittrock (1872).

*M. notabilis* Hassal; *op. cit.*, Transeau, *The Genus Mougeotia*, p. 322.

(Fig. 10.)

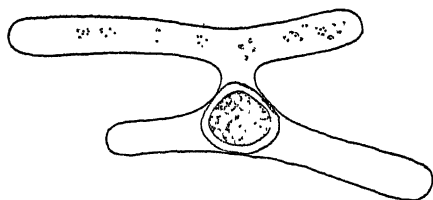


FIG. 10. *Mougeotia tenuis* (Cleve) Witt.

$\times 310$ .

Vegetative cells are  $12\text{--}15\ \mu$  broad. Each cell with a single plate-shaped chloroplast with 5–7 pyrenoids in a single row.

*Conjugation*.—Conjugation scalariform anisogamous. The zygospore is partly in the conjugation canal and partly in the female gametangium, and is cut off by three walls from the gametangia, two walls being situated in the female gametangium, and one of the conjugation canals (Fig. 10). Conjugation between three or more filaments is common. Remains of protoplasm in the form of spots are seen in the arms of gametangia.

Zygospores are rounded in shape and swim about with the remains of the gametangia sticking at their sides. Zygospores  $26\text{--}28\ \mu$  broad. Zygospore wall is composed of two layers only, a smooth and hyaline exospore, and a brownish to bluish mesospore.

*Distribution*.—This species has been reported from North America, Sweden and British Isles.

*Habit.*—Found free-floating mixed with a creeping attached species of *Spirogyra* in a puddle of shallow water in a brownish mass near Rajeh Sultanpur, tehsil Tanda, district Fyzabad, U.P., on 26th November 1936.

9. *Mougeotia floridana* Transeau.

*Op. cit.*, *New Species of Zygnemataceae*, p. 224.

(Fig. 11.)

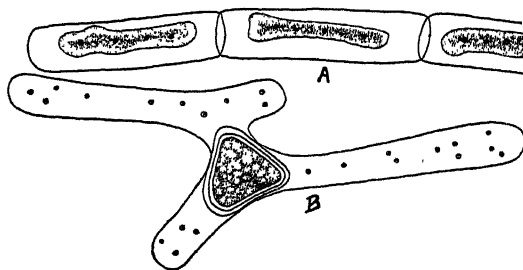


FIG. 11. *Mougeotia floridana* Trans.

A.—Shows vegetative cells with chloroplasts.

B.—Shows a zygospore and remains of protoplasm in the arms of gametangia.

× 310.

Vegetative cells are  $16-20\ \mu$ , 3–8 times as long, each cell with a single chloroplast bearing 5–8 pyrenoids in a single row (Fig. A).

*Conjugation.*—Zygospore is partly in the receptive gametangium and partly in the conjugation canal, triangular ovoid in shape,  $28-32\ \mu$  broad lengthwise. Mesospore smooth. Remains of protoplasm are seen in the form of spots in the arms of gametangia (Fig. B).

*Distribution.*—This species has been so far reported only from the United States of America.

*Habit.*—Free-floating mixed with *Zygnemopsis lamellata* in a jhil near Tanda, district Fyzabad, on 7th February 1937.

*Zygnemopsis* (Skuja) Transeau (1934).

Vegetative cells with plain end walls, two more or less rounded or semi-stellate chloroplasts containing a central pyrenoid, in each cell. In some rare cases one or three to five chloroplasts may be seen in each cell. Reproduction takes place by isogamously produced zygospores, and spindle-shaped aplanospores. Marked geniculation of the mating filaments takes place during conjugation. The gametangia become filled with a dense pectic cellulose colloid, which in most species is secreted in lamellæ, and in a few in a homogeneous mass. The spore wall is composed of three layers, of which the mesospore is yellowish to brownish in colour and is variously sculptured at maturity. The zygospores show a great variety in shape, even in the

same species. The remains of the gametangia persist around the zygospores in the form of horn-like structures. Peculiar triangular zygospores produced as a result of the fusion of the terminal cell of filament with the intercalary cell of another are also seen.

1. *Zygnemopsis indica* Randhawa.  
(Fig. 12.)

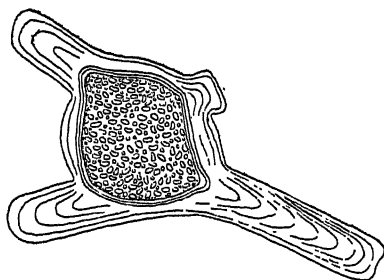


FIG. 12. *Zygnemopsis indica* Randh. shows a spore with its verrucose spore-wall.  
× 440.

Vegetative cells 10–15  $\mu$  broad, 4–5 times as long, each with two semi-stellate chloroplasts with one pyrenoid in each.

*Reproduction*.—Reproduction by means of zygospores and aplanospores. Conjugation canals wide, deposition of shining white pectic cellulose in lamellæ takes place in gametangia. Zygospores globose or quadrately ovoid 36–46  $\mu$  broad excluding the coats of mucilage. Zygospore wall composed of three layers, a thin, smooth, light blue exospore, thick chocolate brown mesospore, and yellowish brown endospore. Spore wall is verrucose (Fig. 12). Triangular zygospores common. Aplanospores spindle-shaped.

*Habit*.—Found free-floating in a yellowish mass in a pond near Hamira, district Jullundar, and V. Shahpur, district Hoshiarpur, Punjab, in the months of February, March and April 1930.

2. *Zygnemopsis splendens* Randhawa.  
(Fig. 13.)

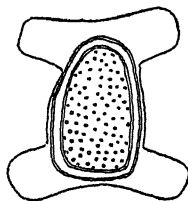


FIG. 13. *Zygnemopsis splendens* Randh. × 440.

Vegetative cells 12–14  $\mu$  broad, 30–40  $\mu$  long each containing two irregularly rounded chloroplasts, each with a central pyrenoid. Septa plain.

*Reproduction*.—Conjugation scalariform, isogamous. The protoplasm secretes a shining white pectic cellulose substance in a homogeneous mass. Zygospores quadrately ovoid or conical,  $26-30\ \mu$  broad and  $40-50\ \mu$  long. Exospore thin, smooth light blue, separated by a wide space filled with yellowish matter from the brownish sinuous mesospore. In fully mature zygospores the spore-wall is scrobiculate with pits  $1\ \mu$  in diameter (Fig. 13).

*Habit*.—Collected from ponds and paddy fields in village Mhow Jadubanspur, Rampur and Rasoolpur, district Fyzabad, U.P., in January, February and in early March 1937.

### 3. *Zygnemopsis lamellata* Randhawa.

(Fig. 14.)

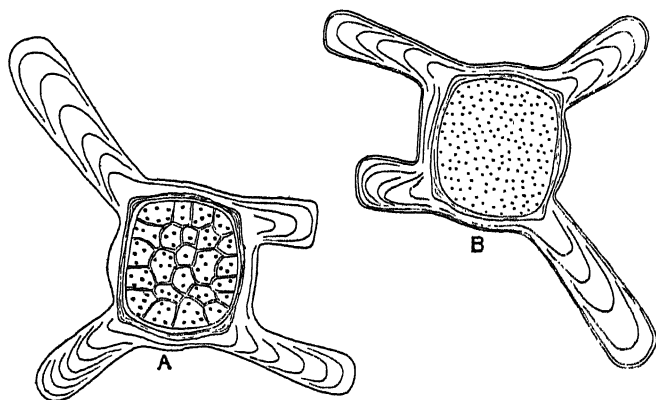


FIG. 14. *Zygnemopsis lamellata* Randh.

A.—Shows a zygospore with reticulations and punctation.

B.—Shows a zygospore with pits.

× 440.

Vegetative cells  $15-18\ \mu$  broad and  $32-42\ \mu$  long, each containing two rounded chloroplasts.

*Reproduction*.—Reproduction by means of zygospores. Conjugation scalariform, isogamous. Lamellæ of white pectic cellulose secreted. Zygospores globose, quadrangular, spindle-shaped or irregular in shape, dark bluish green in colour,  $44-52\ \mu$  broad. The spore-wall bears broad reticulations and is also punctate (Fig A). However, when the shutter of the microscope is fully opened only small pits  $1\ \mu$  in diameter and 3–4 apart are visible (Fig. B). Triangular zygospores also seen.

*Habit*.—Found free-floating in a darkish purpule mass in Tons nadi near tehsil buildings, Akbarpur, district Fyzabad, U.P., in February, March and April 1937 and also in February, March and April 1938.

4. *Zygnemopsis lamellata*. var. *globosum* (emend).

(Fig. 15.)

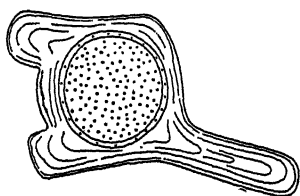


FIG. 15. *Zygnemopsis lamellata*. var. *globosum*.  $\times 440$ .

Vegetative cells  $12-14\ \mu$  broad and  $36-72\ \mu$  long, each containing one or two rounded chloroplasts.

Zygospores typically globose, yellow in colour,  $44-50\ \mu$  in diameter. Originally this form was described as a new species of *Zygnemopsis*. Examination of the zygospores of this form under an oil immersion lens showed that the spore-wall is punctate. This form differs from the type only in the globose form of its zygospores and hence it is reduced to the rank of a variety.

*Habit*.—Found mixed with *Oedogonium* sp. in a water channel near a jhil near Tanda, district Fyzabad, on 28th March 1937.

5. *Zygnemopsis sphærospora* sp. nov.

(Fig. 16.)

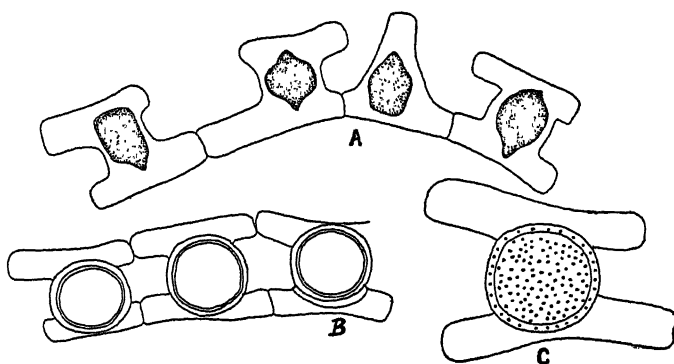


FIG. 16. *Zygnemopsis sphærospora* sp. nov.

A.—Shows immature zygospores.

B.—Shows ripe zygospores.

C.—Shows a ripe zygospore with its punctate spore-wall.

A. and B. are  $\times 310$  and C. is  $\times 440$ .

Vegetative cells are  $14-16\ \mu$  broad and  $45-50\ \mu$  long.

**Reproduction.**—Reproduction takes place by means of zygospores only. Immature zygospores resemble those of *Z. splendens* in shape (Fig. A). Mucilage is secreted in the form of a homogeneous mass. Zygospores are globose, chocolate-brown in colour, and  $34-38\ \mu$  in diameter. Spore-wall is punctate with pits about  $\frac{1}{2}\ \mu$  in diameter (Fig. C).

**Affinities.**—In the absence of lamellation this form resembles *Z. splendens* but differs from it in the shape of spores and structure of spore-wall. From *Z. lamellata* this form differs in the absence of lamellation, brown colour and smaller size of its zygospores.

**Habit.**—Found mixed with *Zygnema* sp. and *Oedogonium* sp. in Thirua nadi near V. Paikolia, tehsil Tanda, district Fyzabad, in a greenish mass on 10th May 1938.

6. *Zygnemopsis Transeauana* sp. nov.

(Fig. 17.)

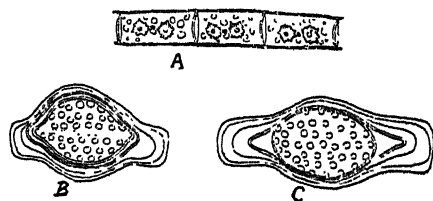


FIG. 17. *Zygnemopsis Transeauana* sp. nov.

A.—Shows a vegetative filament.

B. and C.—Show ripe aplanospores with pitted spore-wall.

A. is  $\times 310$  and B. and C. are  $\times 440$ .

Originally this species was described as an aplanosporic form of *Zygnemopsis lamellata*, as it was found mixed with it. However, later on it was found in pure growths, and the structure of the spore-wall of its mature aplanospores also differs from the zygospores of *Z. lamellata*.

Vegetative cells are  $16-18\ \mu$  broad and  $30-60\ \mu$  long. However the average size is  $16 \times 35\ \mu$  (Fig. A).

**Reproduction.**—Reproduction takes place exclusively by means of aplanospores. In some cases the retreating protoplasm secretes mucilage in a homogeneous mass and in others in the form of lamellæ. Aplanospores are oval (Fig. C) or depressed-globose with an equatorial ridge. Spore-wall is scrobiculate with pits about  $2\ \mu$  in diameter.

**Habit.**—Found in shallow water of a tributary of Tons nadi near Goshaingunja, district Fyzabzd, in February, March and April 1937 and 1938.

7. *Zygnemopsis gracilis* sp. nov.

(Fig. 18.)

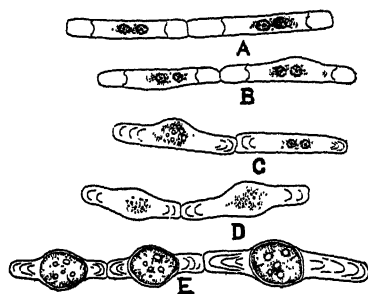


FIG. 18. *Zygnemopsis gracilis* sp. nov.

A.—Shows part of a vegetative filament.

B., C. and D.—Show different stages in the development of aplanospores.

E.—Shows a chain of aplanospores.

All  $\times 310$ .

Vegetative cells are  $6-7\ \mu$  broad and  $40-70\ \mu$  long. Each contains two small more or less rounded chloroplasts surrounded by granular matter (Fig. A).

**Reproduction.**—No conjugation has been noticed in this species though numerous samples were examined and development carefully watched for two months. The only mode of reproduction is by means of aplanospores. In early stages the vegetative cells begin to swell up on one side and the protoplasm becomes granular (Fig. C). The ultimate result is the formation of globose or ovoid aplanospores with granular contents. The retreating protoplasm secretes mucilaginous lamellæ of shining pectic cellulose. No mature aplanospores were discovered, hence it is not possible to describe the nature of spore-wall.

**Affinities.**—This form resembles *Zygnemopsis desmidioides* in the size of its vegetative cells but differs from it in the presence of aplanospores and absence of conjugation. It is much smaller in size as compared with the other aplanosporic species of genus *Zygnemopsis*, and hence it is desirable to describe it as a new species.

**Habit.**—Found free-floating mixed with *Mougeotia sphærocarpa* and *Zygnema Oudhensis* in a purely vegetative condition on 20th February 1938, and producing aplanospores on 12th March 1938 in Achhnaiya nadi near V. Makrahi, tehsil Tanda, district Fyzabad, U.P.

8. *Zygnemopsis Iyengari*, Comb. nov. (*Zygnema Iyengari*) Randhawa.  
(Fig. 19.)

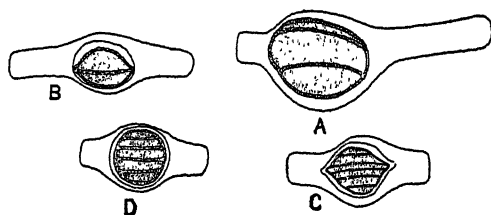


FIG. 19. *Zygnemopsis Iyengari* Randh.

A.—Shows a zygospore laterally.

B., C. and D.—Show aplanospores.

All  $\times 310$ .

Vegetative cells  $12-16\ \mu$  broad,  $60-100\ \mu$  long.

**Reproduction.**—Zygospores globose,  $44-54\ \mu$  in diameter, with a thick bluish green exospore separated by a space filled with light yellowish brown matter from the yellowish brown mesospore. Spore-wall with ridges on surface. Asexual reproduction by barrel-like, or spindle-like aplanospores bearing a number of parallel ridges on surface,  $24-28\ \mu$  in diameter (Figs. B, C and D).

**Habit.**—Free-floating in *Pikia nadi*, near Rajeh Sultanpur, district Fyzabad, U.P., on 15th January 1937.

9. *Zygnemopsis minutum* Randhawa.  
(Fig. 20.)

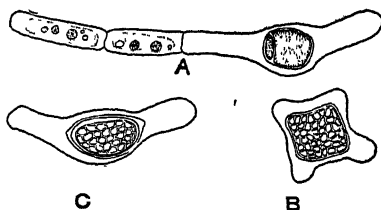


FIG. 20. *Zygnemopsis minutum* Randh.

A.—Shows a vegetative filament with a zygospore.

B.—Shows a ripe zygospore.

C.—Shows a zygospore produced by end to end conjugation.

All  $\times 310$ .

Vegetative cell  $8-10\ \mu$  broad and  $36-46\ \mu$  long. Each cell contains two irregularly rounded chloroplasts. The septa are plain, and the cells have a tendency to dissociate from each other (Fig. A).

**Reproduction.**—Conjugation in this form takes place between free-floating cells which may meet in any position and produce zygospores. Conjugation is isogamous, and H-shaped pairs of conjugating cells may be seen free-floating in water. The protoplasm secretes a shining white pectic cellulose substance in a homogeneous mass. Zygospores are squarish, retain the stumpy arm-like remains of gametangia, are  $22-24\ \mu$  broad excluding the

mucilaginous coat and inclusive of it may be as broad as  $30\ \mu$  and are dark chocolate brown in colour. The spore-wall bears small reticulations on surface and is composed of two layers, a thin and hyaline exospore and a thick dark brown mesospore (Figs. B and C). Three-horned zygosporcs are also seen. Cudgel-shaped bodies described as aplanospores were found to be zygosporcs produced by end to end conjugation of cells (Fig. C).

These are  $18-20\ \mu$  broad and  $18-30\ \mu$  long.

*Habit.*—Free-floating in Tons nadi, Akbarpur, district Fyzabad, U.P., mixed with *Zygnemopsis lamellata* and *Zygnema Oudhensis* in February, March and April 1937 and February, March and April 1938.

10. *Zygnemopsis minutum* var. *crassa*. var. nov.

(Fig. 21.)

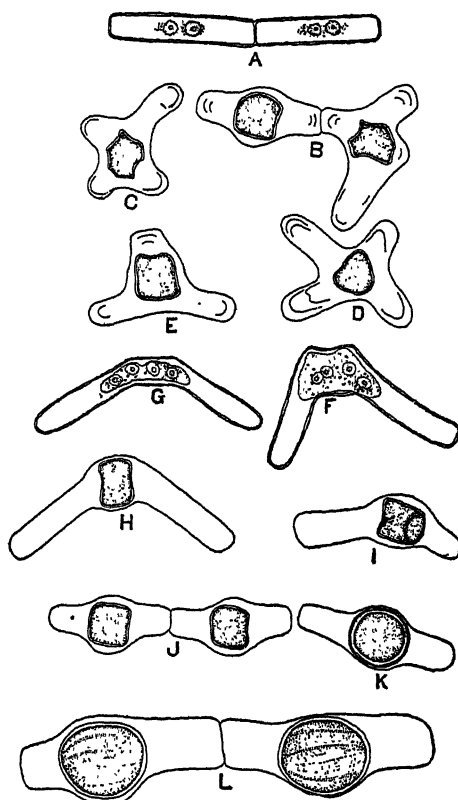


FIG. 21. *Zygnemopsis minutum* var. *crassa*. var. nov.

A.—Shows a vegetative filament.

B, C, and D.—Show 4-horned zygosporcs.

E.—Shows a 3-horned zygosporc.

F. and G.—Show end to end conjugation of free-floating cells.

H. and I.—Show zygosporcs produced as a result of end to end conjugation.

J, K, and L.—Show aplanospores.

All  $\times 310$  excepting L, which is  $\times 440$ .

Vegetative cells are 10–12  $\mu$  broad and 55–70  $\mu$  long.

*Reproduction*.—In this form reproduction takes place by means of zygospores and aplanospores.

*Aplanospores*.—Aplanospores are spindle-shaped bodies, which can be differentiated from the cudgel-shaped zygospores by their smaller size and more or less straight appearance. Each aplanospore develops from a single vegetative cell, and hence seldom exceeds 50–70  $\mu$  in length. Only semi-mature spores light brown in colour were observed (Figs. B, J, K). Their average diameter exclusive of the mucilaginous coat is 18  $\mu$ . A pair of exceptionally big aplanospores with ridges on spore-wall and 24–28  $\mu$  in diameter was also observed (Fig. L).

*Zygospores*.—As conjugation takes place between free-floating dissociated cells, the result is the formation of zygospores with all sorts of shapes. The most peculiar case is when cells meet end to end (Fig. G), and form cudgel-shaped zygospores which look like aplanospores (Fig. H). In fact these peculiar zygospores were originally described as aplanospores. In some cases conjugation canals are given out on one side and V-shaped couples of cells may be seen floating in water (Fig. F). In some cases terminal part of a cell may fuse with the middle part of another resulting in the formation of triangular zygospores (Fig. E). However the majority of zygospores are four-horned bodies like the zygospores of desmids (Figs. B, C and D).

This form differs from the type in the larger size of vegetative cells and also in the peculiar structure of its aplanospores.

*Habit*.—Collected from a small tributary of river Sarju near V. Mubarakpur, district Fyzabad, on 12th March 1938, mixed with *Zygnema Heydrichii*.

*Zygnema* Agardh (1824).

Filamentous habit, each cell with two stellate chloroplasts surrounding a centrally situated nucleus, and each containing a central pyrenoid. Reproduction by zygospores, aplanospores and akinetes. Conjugation isogamous to anisogamous. Zygospore wall of 2–3 layers, mesospore yellow, brown, or blue, smooth or sculptured.

1. *Zygnema Czurdæ* Randhawa.

(Fig. 22.)

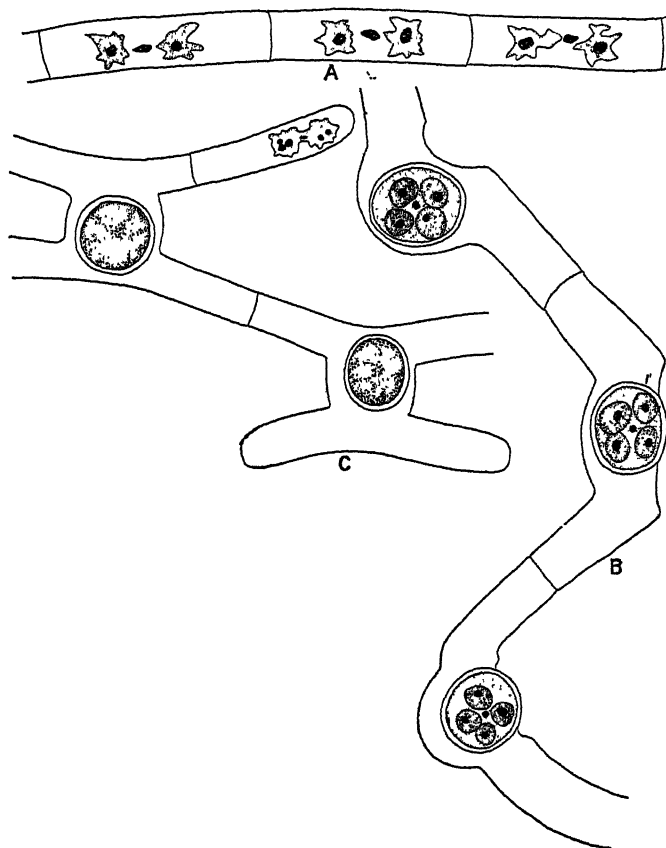


FIG. 22. *Zygnema Czurdæ* Randhawa.

A.—Shows a vegetative filament.

B.—Shows lateral conjugation and geniculation of filaments.

C.—Shows scalariform conjugation.

All  $\times 310$ .

Vegetative cells are  $20-27 \mu$  broad, and  $1\frac{1}{2}$  to 4 times as long. Two more or less rounded chloroplasts with a conspicuous pyrenoid in each, are seen in each cell (Fig. A).

*Reproduction.*—Both lateral and scalariform conjugation have been noticed in this alga.

1. *Lateral conjugation*.—Lateral conjugation is the commonest mode of reproduction in this alga. The zygospore fills the whole of the conjugation canal area, as well as the lower part of the conjugating cells. The zygospores are 30–40  $\mu$  in diameter, and are oval in shape in early stages but later on become rounded. The exospore and mesospore are smooth, while the endospore is slightly sinuous. Distinct geniculation is noticeable in later stages, and the flattened basal part ruptures (Fig. B).

2. *Scalariform conjugation*.—Some of the filaments also show the normal type of scalariform conjugation, with globose zygospores in the conjugation canal. Geniculation is noticeable (Fig. C).

*Habit*.—Found free-floating in a bluish green mass, mixed with a species of *Spirogyra* during the third week of February 1931, in a fresh-water spring at Tahli Sahib, tehsil Dasuya, district Hoshiarpur, Punjab.

2. *Zygnema caeruleum* Czurda.

*Op. cit.*, Czurda, p. 107, *Die Susswasserflora Mitteleuropas*, Heft 9, *Zygnemales*.

Vegetative cells 20–24  $\mu$  broad and 3–4 times as long. Chloroplasts rounded with conspicuous pyrenoids.

*Reproduction*.—Conjugation scalariform. Zygospores in the conjugation canal, completely filling the canal. Zygospores rounded, or ellipsoid in shape 26–36  $\mu$  in diameter. Exospore hyaline, mesospore thick, scrobiculate. Some of the zygospores have mucilaginous coating.

*Habit*.—Found free-floating in a fresh-water stream near Beas during the second week of March 1931, along with *Zygnema giganteum* Randhawa and species of *Spirogyra*.

3. *Zygnema inconspicuum* Czurda, Nov. Nom.

*Op. cit.*, Czurda, *Susswasserflora Mitteleuropas*, Heft 9; and  
Transeau, *Mimeographed Key to the Species of Zygnematales*.

(Fig. 23.)

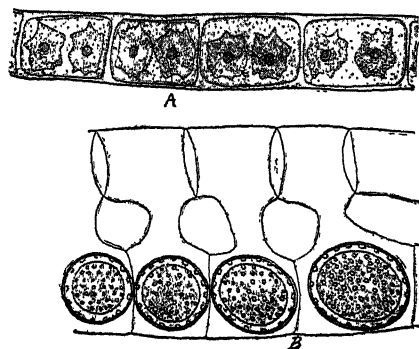


FIG. 23. *Zygnema inconspicuum* Czurda.

A.—Shows a vegetative filament with chloroplasts.

B.—Shows ripe zygospores.

Both  $\times 310$ .

Vegetative cells  $30\text{--}36\ \mu$  broad, and  $40\text{--}50\ \mu$  long, each with two massive stellate chloroplasts (Fig. A).

*Reproduction*.—Anisogamous conjugation. Zygospores globose to oval in shape  $30\text{--}48\ \mu$  in diameter. Zygospore wall composed of two layers, a thin smooth and brown exospore, and a thick chocolate brown scrobiculate mesospore. Pits about  $2\ \mu$  in diameter. Fruiting cell not swollen (Fig B).

This alga differs from the type in the presence of globose as well as oval zygospores, the smaller size of the zygospores and their pits.

*Habit*.—Collected from a slowly flowing nadi near Tanda, district Fyzabad, U.P., on 10th January 1937.

4. *Zygnema cyanosporum* Cleve (1868).

*Op. cit.*, Czurda, *Susswasserflora Mitteleuropas*, Heft 9.

Vegetative cells are  $20\text{--}22\ \mu$  broad and 3–4 times as long. Chloroplasts two, rounded in each cell with the nucleus in the middle.

*Conjugation*.—Conjugation is scalariform with the zygospore in the conjugation canal. Zygospores rounded in shape. Zygospore wall is composed of two layers only, a thick, hyaline exospore, and a bluish and smooth mesospore. Zygospores are  $26\text{--}30\ \mu$  in diameter. Conjugation between three or more filaments is quite commonly seen.

*Habit*.—Found free-floating in a yellowish green mass in a fresh-water stream near Makrahi, district Fyzabad, U.P., during the second week of November 1936.

5. *Zygnema Heydrichii* Schmidle. var. *indicum*. var nov.  
(Fig. 24.)

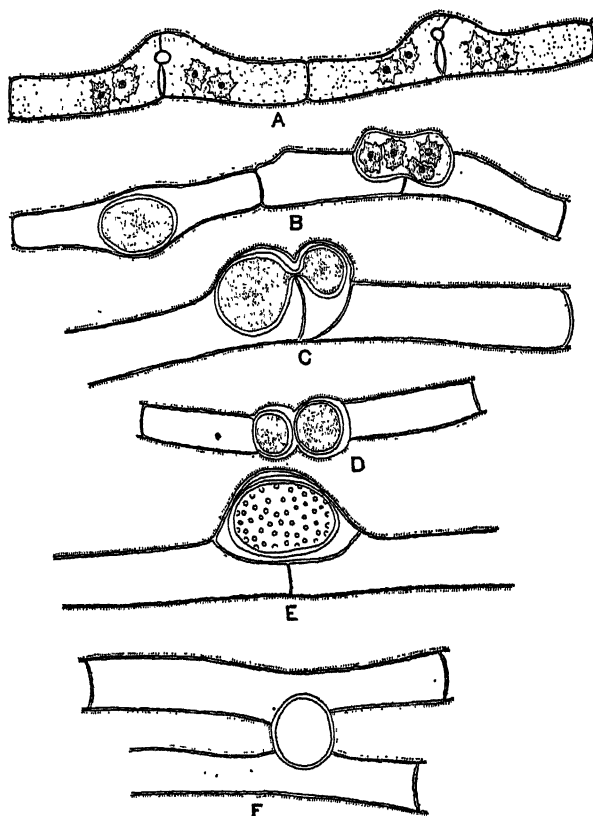


FIG. 24. *Zygnema Heydrichii* Schmid. var. *indicum* var. nov.

A.—Shows an early stage in lateral conjugation.

B.—Shows immature zygospores.

C. and D.—Show azygospores or parthenospores.

E.—Shows a ripe spore produced by lateral conjugation.

F.—Shows scleriform conjugation.

All  $\times 440$  excepting D. and F. which are  $\times 310$ .

Vegetative cells are  $18-22\ \mu$  broad and  $50-94\ \mu$  long. Each cell has two more or less stellate chloroplasts surrounding a centrally situated nucleus. The protoplasm of the cells is densely granular. On the exterior the cells are surrounded by a hyaline mucilaginous covering (Fig. A).

*Reproduction.*—Unlike the type this alga reproduces both by lateral and scleriform conjugation. A. *Lateral Conjugation.* However, the predominant mode of conjugation is lateral. The neighbouring cells give out tube-like protuberances which ultimately meet forming a dome-like structure (Fig. B).

The dome-like space containing the zygospore is cut off by a partition wall from the remaining part of the conjugating cells (Fig. E). In one case it was noticed that the partition wall was laid obliquely and the gametes instead of meeting had independently developed into parthenosporic bodies joined together by a very narrow isthmus (Fig. C). Looked at laterally these partheonospores with their swollen walls gave the filament the appearance of an *Oedogonium* (Fig. D). In some cases the partition wall is obliterated entirely and the zygospore appears to lie in the swollen middle part of the cells, while in some cases the vertical septum separating the cells may be seen. Zygospores are greenish blue to dark blue in colour, and from oval to reniform in appearance. Zygospores are  $22-27\ \mu$  broad and  $31-37\ \mu$  long. Mesospore is scrobiculate with pits about  $2\ \mu$  in diameter.

*B. Scleriform Conjugation.*—This mode of conjugation is comparatively very rare. Zygospores were on the average  $21\ \mu$  broad and  $25\ \mu$  long and agreed in all details with those produced by lateral conjugation (Fig. F).

*Affinities.*—This form resembles *Z. Heydrichii* Schmidle in the structure of its zygospores and in their peculiar location when produced laterally, and differs from the type in the presence of scalariform conjugation, more numerous pits and blue colour of spore-wall. Hence it is desirable to describe this as a new variety of *Z. Heydrichii*. A form described as *Z. gangeticum* sp. nov. by Rao is presumably a laterally reproducing form of this species. He described that form which resembles the present one in many features, as having smooth-walled spores. This may be due to his having observed only immature spores.

*Habit.*—Found mixed with *Zygnomopsis minutum* var. *crassa* in a freshwater stream near V. Mubarakpur, district Fyzabad, U.P., on 12th March 1938.

6. *Zygnema mucigena* sp. nov.

(Fig. 25.)

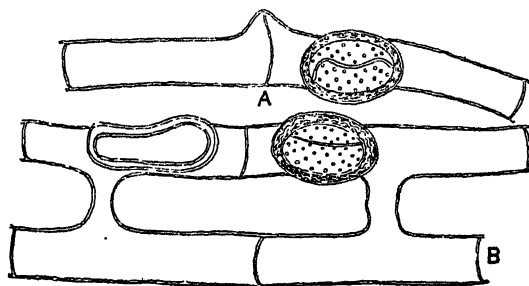


FIG. 25. *Zygnema mucigena* sp. nov.

A.—Shows lateral conjugation.

B.—Shows scalariform conjugation.

Both  $\times 440$ .

Vegetative cells  $12-14\ \mu$  broad,  $50-100\ \mu$  long, each with two more or less globose chloroplasts.

*Conjugation*.—This form reproduces both by lateral and scalariform conjugation, but the former mode of conjugation is very common. In filaments reproducing by means of scalariform conjugation, the conjugation canals are very much elongated, and zygospores are found in one of the gametangia (Fig. B).

The zygospores are dark bluish green in colour, are oval in shape,  $20-22\ \mu$  broad, and  $30-36\ \mu$  long. Mesospore is thick, greenish bluish and prominently pitted. There are 5-6 rows of pits, which are about  $1-1\frac{1}{2}\ \mu$  in diameter and are  $3-4\ \mu$  apart (Figs. A and B).

*Affinities*.—This species resembles *Z. Carteri* Czurda in the size and shape of vegetative filaments and zygospores; but differs from it in its anisogamous scalariform conjugation.

*Habit*.—Found free-floating in a light blue mucilaginous mass in Rampur jhil tehsil Akbarpur, district Fyzabad, on 15th December 1937.

7. *Zygnema chalybdospermum* Hansg.

*Op. cit.*, Czurda, *Zygnemales*, Heft 9 in *Susswasserflora, Mitteleuropas*.

Vegetative cells  $20-27\ \mu$  thick, 1-3 times as long. Chloroplasts typically stellate, each with one pyrenoid.

Conjugation scalariform. Zygospores lodged in the gametangia more or less rounded in shape  $28-30\ \mu$  broad and  $30-32\ \mu$  long.

*Habit*.—Free-floating in a pond at V. Jhingran, district Hoshiarpore, during the middle of March 1930. Also collected near Hamira from a pond, about the middle of April 1930.

8. *Zygnema collinsianum* Transeau.

(Fig. 26.)

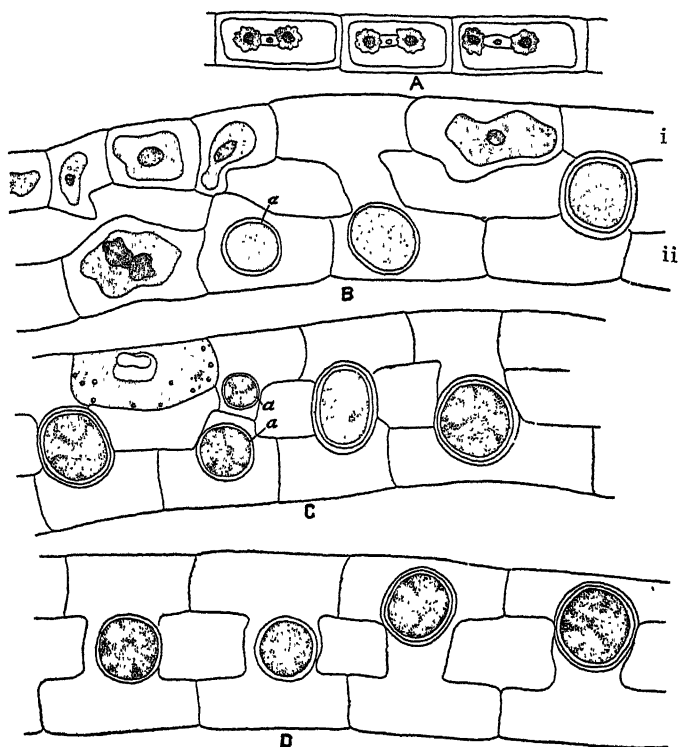


FIG. 26. *Zygnema collinsianum* Transeau.

A.—Shows a vegetative filament.

B, C. and D.—Show different parts of the same pair of conjugating filaments with zygospores and azygospores (*a*).  
× 310.

Vegetative cells 18–24  $\mu$  broad, and  $2\frac{1}{2}$ – $3\frac{1}{2}$  times as long. Chloroplasts two in each cell, rounded, each with a conspicuous pyrenoid (Fig. A).

*Reproduction*.—This remarkable species of *Zygnema* shows a peculiar mode of conjugation which hovers between isogamy and anisogamy as in *Z. giganteum*. In the same filament we see isogamously produced zygospores lodged in the conjugation canal, or anisogamously produced zygospores lying in one of the cells. Figs. B, C and D show different parts of the same pair of conjugating filaments.

The sexuality of this alga is very much unsettled, cells of the same filament producing isogamous gametes, passive female gametes, and more active male gametes.

Zygospores are rounded in shape and are  $24-36\ \mu$  in diameter. Only immature spores were observed.

*Azygospores*.—Azygospores may also be seen in some of the cells. In Fig. B we see that the contents of one of the cells of filament *ii* have rounded off and developed a thick wall, before the establishment of a continuous conjugation canal. In Fig. C we see that both of the gametes in opposite cells in one case, have developed into azygospores.

*Habit*.—Found free-floating mixed with a species of *Oedogonium* in a fresh-water lake near Baskhari, tehsil Tanda, district Fyzabad, U.P., in the first week of December 1936.

9. *Zygnema giganteum* Randhawa.

(Fig. 27.)

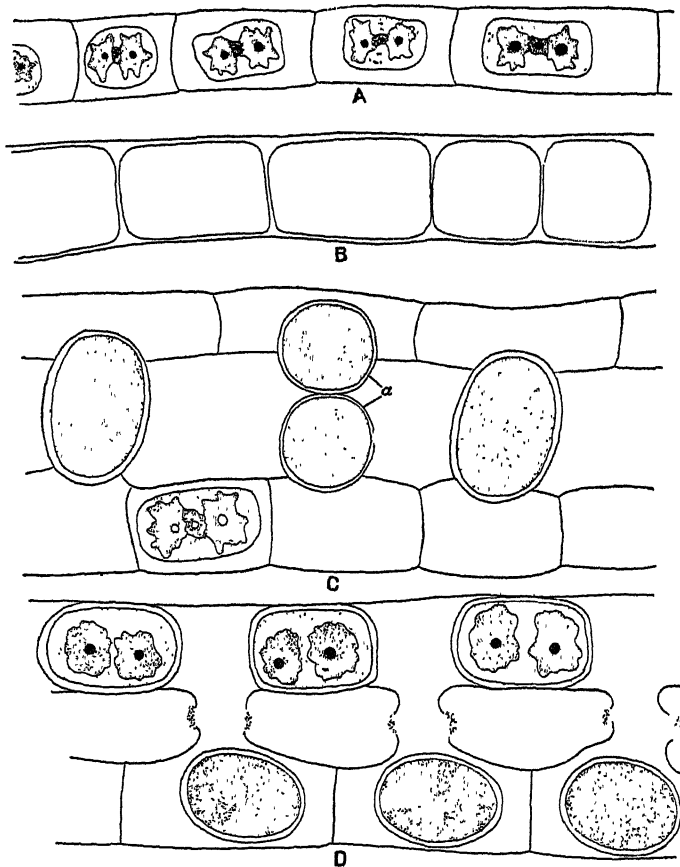


FIG. 27. *Zygnema giganteum* Randh.

A.—Shows a vegetative filament.

B.—Shows a chain of akinetes.

C.—Shows isogamous conjugation with zygospores and azygospores (a).

D.—Shows anisogamous conjugation.

× 330.

Vegetative cells are  $38-48\ \mu$  broad and  $1\frac{1}{2}-2\frac{1}{2}$  times as long. In thinner filaments, the chloroplasts show a typically stellate structure each with a conspicuous pyrenoid (Fig. A). In bigger filaments the chloroplasts are loaded with starch granules, and the stellate structure of the chloroplasts is obscured, and they appear to be more or less rounded in appearance. Cell wall is fairly thick as compared with other species of *Zygnema*.

*Reproduction*.—Both sexual and asexual modes of reproduction have been noticed in this alga.

(i) *Asexual Reproduction*.—Asexual reproduction takes place by means of brick-shaped akinetes which develop orange-coloured thick walls, and are  $36-45\ \mu$  broad and  $54-96\ \mu$  in length. These may be seen singly, in rows of twos or threes, and in later stages in long chains of many (Fig. B).

(ii) *Sexual Reproduction*.—In some filaments zygospores are found in the conjugation canals, and in others in the conjugating cells, the conjugation being isogamous and anisogamous in the same alga.

(a) *Anisogamous Conjugation*.—This type of reproduction is quite common in most filaments. The male filaments sometimes show an alternation of cells which produce male gametes, and vegetative cells, in which the chloroplasts are surrounded by a shining mucilaginous material and thick walls.

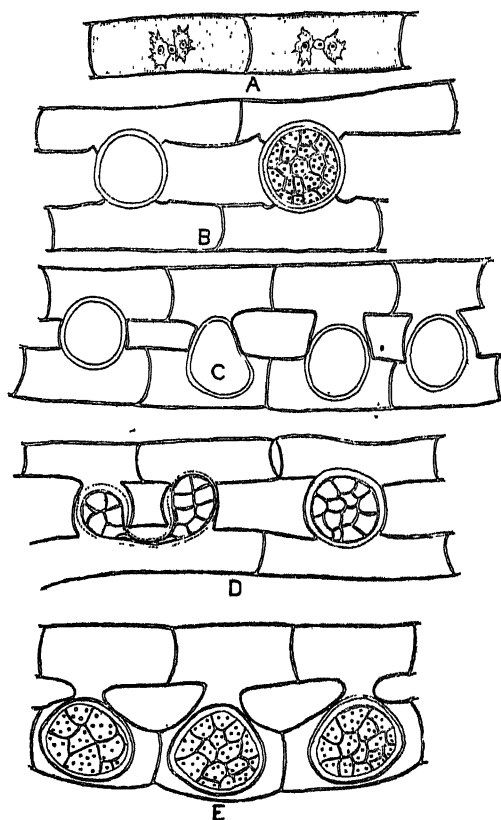
The zygospores are  $42-46\ \mu$  broad and  $50-58\ \mu$  long, and are oval in shape. The zygospore wall is composed of two layers only, a thick hyaline and smooth exospore, and a thin, light blue, and smooth mesospore. The ripe zygospores are orange-coloured in appearance like the parthenospores (Fig. D).

(b) *Isogamous Conjugation*.—This is the commoner mode of reproduction in this alga. Zygospores are typically oval in appearance, and project partly into the gametangia, completely filling the conjugation canals at the same time. Zygospores produced by isogamous conjugation are longer than those produced by anisogamous conjugation, being  $70-75\ \mu$  long. Azygospores also may be seen (Fig. C).

*Habit*.—This alga was found free-floating along with *Zygnema caeruleum* in Siah Baeen, a perennial fresh-water stream in Kapurthala State, Punjab, during the second week of March 1931.

10. *Zygnema Oudhensis* sp. nov.

(Fig. 28.)

FIG. 28. *Zygnema Oudhensis* sp. nov.

A.—Shows a vegetative filament.

B. and D.—Show isogamous conjugation.

C.—Shows unstable location of zygospores.

E.—Shows anisogamous conjugation.

Sculpturing of spore-wall is shown in B. and E. All  $\times 310$ .

Vegetative cells are  $22-34\ \mu$  broad and  $52-70\ \mu$  long, and each cell contains two stellate chloroplasts (Fig. A).

*Conjugation.*—This alga reproduces by means of both isogamous and anisogamous conjugation. While some filaments may be conjugating exclusively in an isogamous fashion (Fig. B), others may show exclusive anisogamy (Fig. E), while still others hover between isogamy and anisogamy (Fig. C). Zygospores are globose to conical in shape, are  $34-46\ \mu$  in diameter, and are dark greenish blue in colour. The spore-wall bears broad

reticulations on its surface and is also punctate with pit  $\frac{1}{2}\mu$  in diameter (Figs. B and E).

In one peculiar case two cells were seen conjugating with the same cell. The spores resulting from this peculiar union were much smaller in size compared with normal spores, and were joined together by an isthmus (Fig. D).

*Affinities.*—This alga resembles *Z. Chungii* Li in the structure of its spores, but differs from it in the presence of isogamy with anisogamy and the size and shape of spores.

*Habit.*—This is the commonest species of *Zygnema* in Oudh, and was collected from January to March in 1937 and 1938 in Fyzabad, Azamgarh, and Gonda districts.

11. *Zygnema terrestris* sp. nov.

(Fig. 29.)

This remarkable species of *Zygnema* was collected by the author from a field lying fallow at the close of the rainy season in the last week of September 1937. It was observed in the form of a light-green felt-like covering in a shaded part of a field, near village Mamrezpur, district Fyzabad, resembling *Vaucheria sessilis* in its external appearance. On further exploration numerous fields were found full of this alga and in more exposed parts the alga appeared purplish in colour. Patches of a species of *Microcoleus* were also seen along with it.

*Vegetative Structure.*—The filaments were usually intricately intertwined, and consist of two parts, which are rather sharply defined. Of these the subaerial part consists of short cells usually 18–24  $\mu$  broad and 36–60  $\mu$  long, each with two spherical chloroplasts each of which bears a massive pyrenoid and with a nucleus in the middle (Fig. A). In some cases the outline of chloroplasts is obscured due to abundance of starch granules and other food material. The lower cells of the subaerial part contain smaller chloroplasts more irregular in outline and fewer granules. The cells of the underground part are very much elongated and in some cases may be as long as 250  $\mu$ . Their chloroplasts are very much attenuated, and the distal part of the lowermost cell is usually expanded (Fig. B). No branching of cells was seen in the underground part. Apparently the function of the subterranean cells is that of fixation like rhizoids. The subterranean part rarely consists of 4–5 cells, but what is lacking in numbers is made up in length of cells.

*Reproduction.*—Even in the first week of October 1937 the alga was seen conjugating freely and also producing numerous aplanospores. Reproduction by means of aplanospores appears to be commoner than by conjugation.

11. *Zygnema terrestris* sp. nov.  
(Fig. 29.)

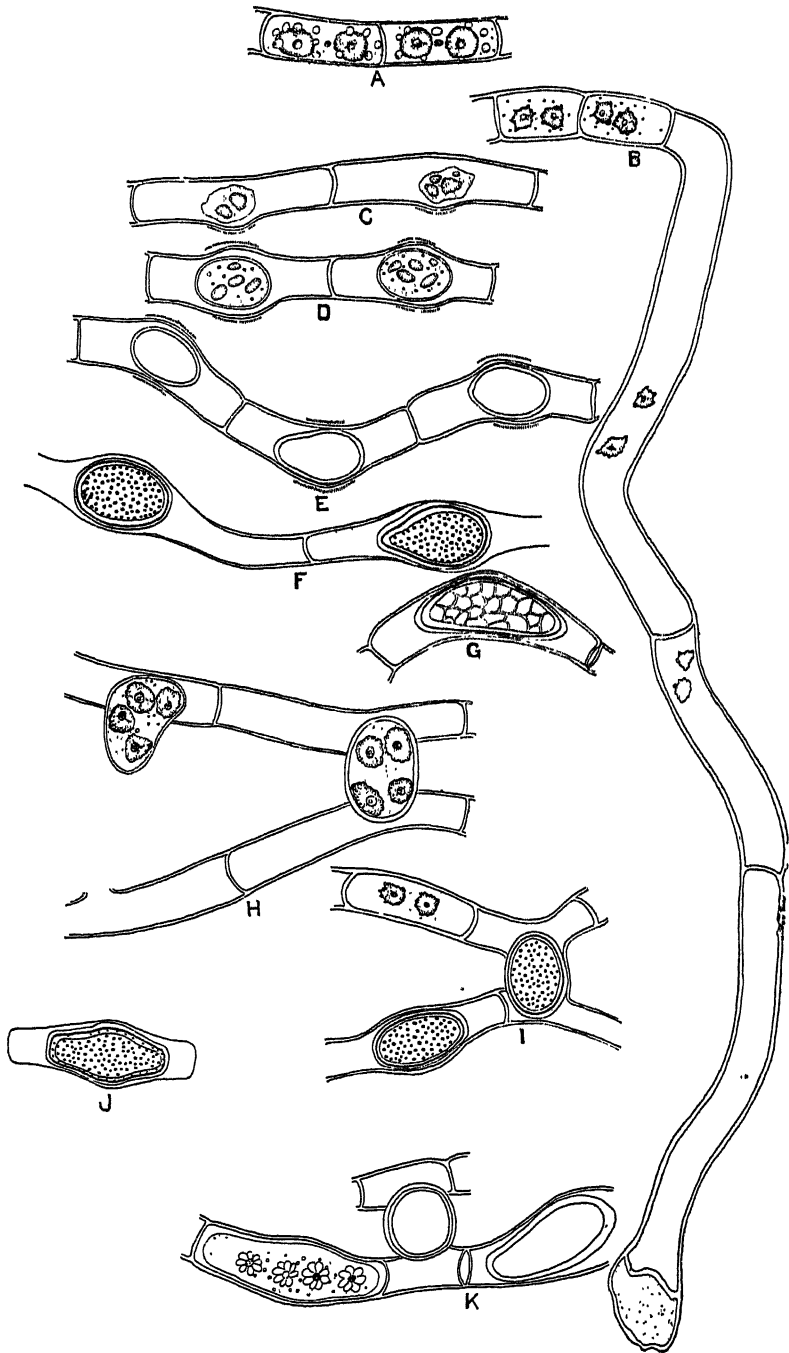


FIG. 29. *Zygnema terrestris* sp. nov.

- A.—Subaerial part of a filament showing two cells with chloroplasts, nuclei and starch granules.
- B.—Lower part of a filament showing 2 subaerial cells and 3 underground rhizoidal cells.
- C.—An early stage in the formation of aplanospores.
- D. and E.—Later stages in the formation of aplanospores. In Fig. E. note geniculation of filaments.
- F.—Shows mature aplanospores or laterally produced zygospores. Note the punctation of spore-wall.
- G.—An aplanospore showing reticulations on spore-wall.
- H. and I.—Show different stages in scalariform conjugation.
- J.—Shows a barrel-like aplanospore.
- K.—Shows fusion of contents of two neighbouring cells laterally. All  $\times 310$ .

*Aplanospore-formation*.—In early stages of aplanospore-formation, cells show a slight swelling on one side (Fig. C). Later on the cells swell up on both sides, and a gelatinous mucilaginous cover is seen surrounding the swollen part (Figs. C, D and E). In some filaments geniculation of cells, giving the filaments a zig-zag appearance is also seen (Fig. E). The mature aplanospores are of dark greenish blue colour, and are of diverse shapes. Some are pyramidal (Fig. G), some are barrel-shaped (Fig. J), but oval shape with slight modification preponderates. Spore-wall consists of three layers, of which the mesospore is the thickest and is dark greenish-blue in colour, while the exospore and endospore are thinner and are light slate-blue in colour. The aplanospores are  $30-34\ \mu$  broad and  $36-65\ \mu$  long, but the average size is  $36 \times 46\ \mu$ . The spore-walls are punctate with pits  $\frac{1}{2}\ \mu$  in diameter and  $3\frac{1}{2}-5\ \mu$  apart (Figs. F and J). Reticulations are also seen on the spore-wall when light is shut out by closing the shutter of the microscope (Fig. G).

*Conjugation*.—Isogamous scalariform conjugation seems to be the predominant mode of conjugation in this alga. However, possibilities of lateral conjugation are not altogether excluded. In one case a swollen cell was seen with four pyrenoids, each of which was surrounded by a ring of starch granules (Fig. K). This could only result from a lateral fusion of gametes. So it is quite possible that some spores which appear like aplanospores, may have resulted in some cases from lateral conjugation. The occurrence of some spores in very long cells also shows the possibility of such a lateral fusion.

The immature zygospores are usually very broad, and not only completely fill the conjugation canals, but also extend into the gametangia. No remains of protoplasm were seen in the gametangia and the zygospores are not cut off by any walls from the gametangia (Figs. H, I and K). The zygospores are oval in shape, are  $28-38\ \mu$  broad and  $36-54\ \mu$  long. In structure they resemble the aplanospores in all details.

*Affinities.*—In its terrestrial habit the shape and structure of chloroplasts, thick cell wall and the abundance of aplanospores, this alga resembles the species of *Zygogonium*. In fact, when first discovered, the author was under the impression that he was dealing with a species of *Zygogonium*. The resemblance of its chloroplasts with those of *Zygogonium sinense* Jao is well-marked. However, the absence of cytoplasmic residue from the gametangia precludes its relationship with *Zygogonium*. From the other known species of *Zygnema*, this alga differs in its habit, shape of its chloroplasts and abundance of aplanospores formation. This species forms a connecting link between the species of *Zygnema* and *Zygogonium*. While in the structure of its chloroplasts, its thick cell walls, terrestrial habit and abundance of aplanospores it resembles the species of *Zygogonium*, it shows a mode of scalariform conjugation with the formation of zygospores from the entire protoplasm of gametangia as in most species of *Zygnema*.

So far as the difference in the structure of cells, chloroplasts and presence of aplanospores between the species of *Zygnema* and *Zygogonium* are concerned, these are effectively bridged by *Zygnema terrestris*. So the only difference which separates *Zygogonium* from *Zygnema* lies in the formation of zygospores and aplanospores from a part of the protoplasm only and the resultant deposit of protoplasmic residue in the gametangia. However, this difference is very material for it means an evolutionary advance similar to that shown by species of *Mougeotia* as compared with those of *Debarya*.

*Habit.*—Found growing in moist fields lying fallow on the sides of a fresh-water stream near V. Mamrezipur, district Fyzabad, U.P., India, in late September, October and early November 1937.

## 12. *Zygnema* sp.

(Fig. 30.)



FIG. 30. *Zygnema* sp.  
Mark the akinete-like cells.

× 440.

Only sterile filaments of this species were seen. However these are of interest, as each protoplast is surrounded by thick lamellated cell-walls. It is probable that these cells function as akinetes.

Cells are 20–22  $\mu$  broad and 14–20  $\mu$  long.

*Habit.*—Found mixed with *Zygnemopsis sphaerospora* in *Thirua nadi* near Tanda, on 11th May 1938.

# SORGHUM—SIZE RELATIONSHIPS OF SEED, EMBRYO, SEEDLING AND THE FIRST SEEDLING LEAVES.

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THE range of variation of seed size in sorghum varieties is very wide (Fig. 1). This is well brought out in this study of six representative varieties ranging from the wild *Sorghum halepense* with the smallest seeds to *Sufra* the grain sorghum with the biggest seeds. A hundred seeds from each of the varieties were measured with a vernier calipers for the three dimensions: (1) the length measured from the hilum to the apex, (2) the maximum breadth and (3) the maximum thickness. The weight of the seed was also recorded. Table I sets forth the averages of these measurements.

TABLE I.  
*Sorghum—Seed Dimensions and Weight.*

Variety	Length	Breadth	Thickness	Weight
	mm.	mm.	mm.	mgm.
<i>S. halepense</i> (Linn.), Pers. ..	2.60	1.63	1.11	2.56
<i>S. margaritifera</i> , Stapf. ..	3.60	2.60	1.60	8.36
<i>S. Roxburghii</i> var. <i>hians</i> , Stapf. ( <i>Talai Virichan Cholan</i> ) ..	4.00	3.10	2.30	17.57
<i>S. durra</i> , Stapf. var. <i>coimbatorensis</i> (Burkill), Snowden ( <i>Peria Manjal Cholan</i> ) ..	4.60	3.70	2.70	28.11
<i>S. caudatum</i> var. <i>feterita</i> , Stapf.	5.01	4.82	3.08	41.99
<i>S. durra</i> var. <i>niloticum</i> (Koern) Snowden ( <i>Sufra</i> ) ..	5.90	5.81	3.25	52.01

In order to determine the relationship between seed size and embryo size, the seed was cut lengthwise across its breadth in the plane of its thickness, which is the most convenient manipulation for laying bare the embryo. The maximum length and breadth of the embryo were measured in  $\mu$  under a microscope. Table II gives the correlations between the dimensions of the seed and those of the embryo.

TABLE II.

*Correlation between Embryo and Seed Dimensions.*

First Correlate Embryo	Second Correlate Seed		
	Length	Breadth	Thickness
Length .. ..	.98	.99	.94
Breadth .. ..	.95	.97	.91

There is a high correlation between the seed and embryo dimensions. The bigger the seed, the larger is the embryo.

In the course of various germination tests conducted in sorghum the remarkable variations in the seedling size was evident. To what extent a seedling depends for its size on the seed producing it, can be estimated by correlating the seedling size with the seed weight. The seed size may be taken as being approximately proportional to the product length  $\times$  breadth  $\times$  thickness ( $= V$ ).  $V$  is highly correlated with the weight of the grain ( $r = .97$ ). The sorghums vary in the details of their shapes and in view of the above high correlation between  $V$  and weight of seed, it is easier to relate seedling characters to the weight of the seed.

A number of measurements suitable for defining the size of seedling were recorded in 100 seedlings of each of the typical varieties in Table I. The seedlings were measured when 10 days old. Table III presents the relation between the weight per seed and the seedling measurements.

On the level of significance  $P = .05$ , the critical correlation coefficient is .81.

It is seen in Table III that the heavier the seed, the greater is the vigour of the seedling as manifested in the thickness of the stem, the coleoptile

TABLE III.

*Correlation between Seed Weight and Measurements of Ten Days Old Seedlings.*

Correlates	<i>r</i>
Weight per seed and thickness of stem (between the first and second leaves) .. .. .	.97
„ „ „ „ length of coleoptile .. .. .	.96
„ „ „ „ total height of seedling .. .. .	.96
„ „ „ „ height of first leaf-sheath .. .. .	.88
„ „ „ „ height of second leaf-sheath .. .. .	.88

length, and the height (Fig. 2). Table IV brings out the fact that as the correlation between seed weight and leaf width diminishes from the first to the third leaf, that between seed weight and leaf length increases to significance. The influence of the seed weight is clearly manifested in the width

TABLE IV.

*Correlation between Seed Weight and Measurements of the First Three Seedling-Leaves.*

Leaf No. reckoned from below	Correlation between seed weight and	
	Leaf breadth	Leaf length
1 ..	.91	.33
2 ..	.88	.44
3 ..	.75	.92

of the first leaf, and declines towards the third leaf to non-significance. The seed weight does not tell upon the length of the leaf initially, but is felt at the third leaf stage.

The three dimensions of the seed considered here are all highly correlated each with the other as seen in Table V.

TABLE V.  
*Correlations between Seed Dimensions.*

Correlates	<i>r</i>
Seed length and breadth ..	.99
Seed breadth and thickness ..	.96
Seed thickness and length ..	.97

Despite the high positive correlations a difference in the rate of variation of the length, breadth and thickness of the seed causes a variation in the shape of the seed grading from *S. halepense* to *Sufra* (Table VI). The  $\frac{\text{length}}{\text{breadth}}$  ( $=v$ ) index, diminishes from 1.60 in *S. halepense* to 1.02 in *Sufra*.

TABLE VI.  
*Seed Shape.*

Variety	$\frac{\text{Length}}{\text{Breadth}}$	$\frac{\text{Length} \times \text{Breadth}}{\text{Thickness}}$	V
<i>S. halepense</i> (Linn.), Pers. ..	1.60	3.82	4.70
<i>S. margaritifera</i> , Stapf. ..	1.38	5.85	14.98
<i>S. Roxburghii</i> , var. <i>hians</i> , Stapf. ( <i>Talai Virichan Cholan</i> ) ..	1.29	5.39	28.52
<i>S. durra</i> , Stapf. var. <i>coimbatorensis</i> (Burkill), Snowden ( <i>Peria Manjal Cholan</i> ) ..	1.24	6.30	45.95
<i>S. caudatum</i> var. <i>feterita</i> , Stapf.	1.04	7.84	74.38
<i>S. durra</i> var. <i>niloticum</i> (Koern), Snowden ( <i>Sufra</i> ) ..	1.02	10.55	111.41

The correlation between seed size as already defined by V and the index  $i$  is  $-.92$ . As the seed size increases the breadth tends to equal the length. It is also seen that relatively to the cross-section length  $\times$  breadth, the thickness of the seed diminishes as the seed size increases. If  $\frac{\text{length} \times \text{breadth}}{\text{thickness}}$  is taken as a measure of the flatness of the seed the correlation between the size of the seed and the flatness of the seed is  $.97$ . The larger seeds are comparatively flatter than the smaller ones.

Table VII presents the correlations between the dimensions of the seed and the length and breadth (maximum) of seedling leaves.

TABLE VII.

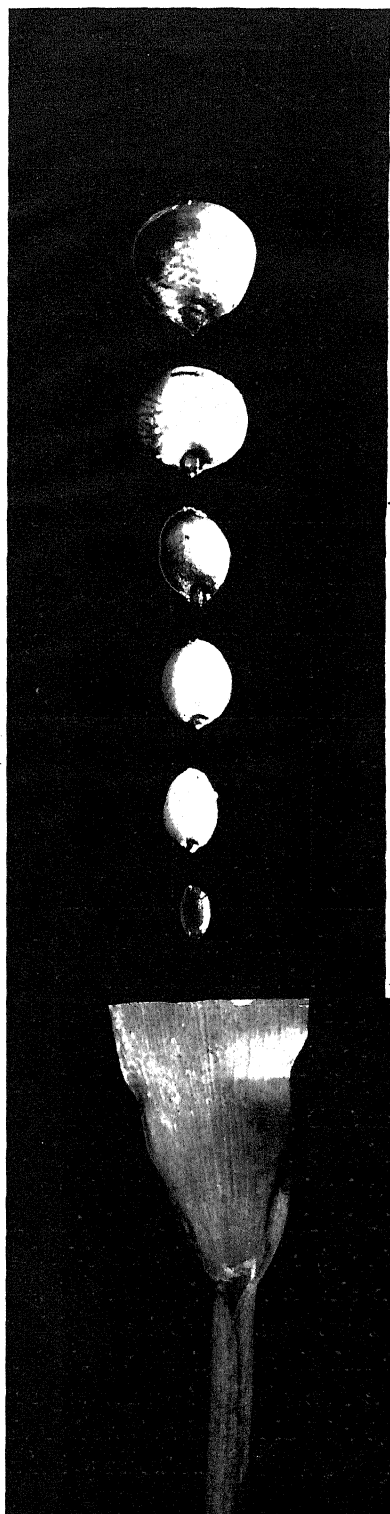
Leaf number reckoned from below	Correlation between seed thickness and		Correlation between seed breadth and		Correlation between seed length and	
	Leaf breadth	Leaf length	Leaf breadth	Leaf length	Leaf breadth	Leaf length
1 ..	.99	.48	.94	.36	.94	.46
2 ..	.95	.51	.85	.42	.86	.49
3 ..	.89	.94	.77	.95	.78	.98

The seed size as defined by V is highly correlated with seed weight ( $r = .97$ ). So, the influence of the seed weight on the seedling leaf dimensions will be expected to reappear in the relationship between the size and dimensions of the seed and those of the leaf. Table VII shows that this expectation is realised. As the correlation under the column breadth diminishes from the first to the third leaf, that under length increases to significance. There is a significant and high correlation between the seed dimensions and the width of the first two seedling leaves (Fig. 3), and between the seed dimensions and the length of the third leaf (Fig. 2). From this third leaf upward, the leaf-blades lengthen out, out of all proportion to their width until the plant is provided with a safe and efficient apparatus for photosynthetic activities. Of the many structural changes that take place in the leaf to enable it to discharge this function efficiently, the most noticeable are a more pronounced midrib and a more elaborate ligular and auricular area. This elaboration is seen in Fig. 4, in which the ligular area of the first three leaves is presented.

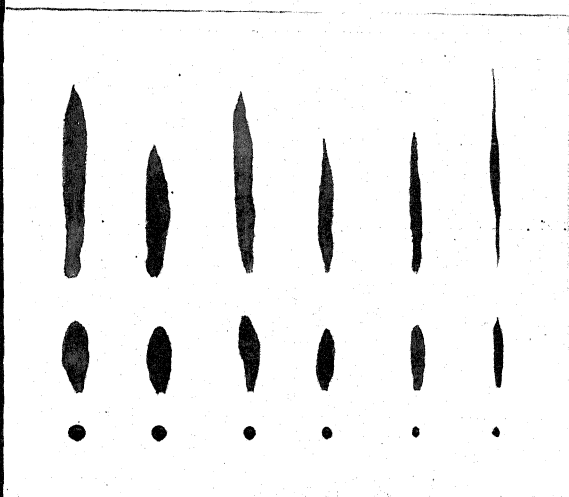
*Summary*

The seeds of sorghum vary very much in their size. Seed size (length  $\times$  breadth  $\times$  thickness) is highly correlated to seed weight. The larger the seed the bigger is its embryo, and the bigger the seedling that grows from it. In six varieties typical of different seed sizes the relationship between seed and seedling leaf measurements have been pursued. The first two seedling leaves are abnormal, in shape, being much wider than long. In these two seedling leaves the width of the seedling is highly correlated with seed size. In the third seedling leaf the length of the leaf is beginning to be influenced by seed size. In this third leaf the midrib and ligular and auricular structures attain their full development.

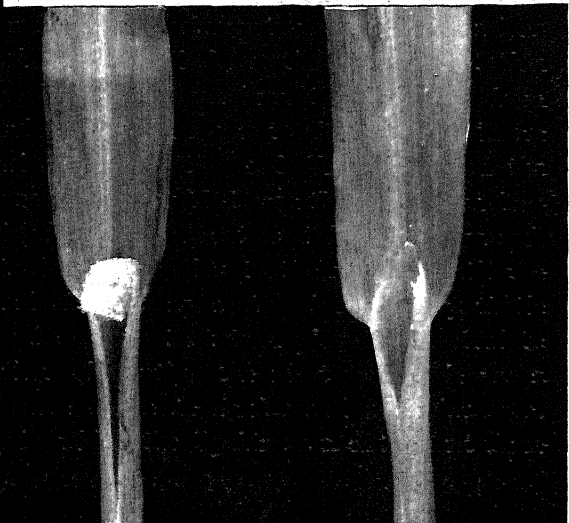
1



2



3



4

FIG. 1. Typical seed sizes of Sorghum varieties ( $\times 3$ ).

FIG. 2. Ten days old seedlings from these seeds.

FIG. 3. Seeds and first and second seedling leaves. Note the decrease in width.

FIG. 4. First, second and third seedling leaf with elaboration of midrib and ligular and auricular appendages.



# THE MYXOPHYCEÆ OF THE ORISSA PROVINCE, INDIA—I.\*

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DURING the earlier half of November 1936, the writer made a fairly rich collection of algæ in the Province of Orissa, mainly from Berhampur,<sup>1</sup> Puri<sup>2</sup>, Cuttack<sup>3</sup> and Khurda Road<sup>4</sup>. The investigation of these algæ is being done in parts and they will be recorded in a series of papers.

But for the few forms recorded by Biswas,<sup>5</sup> there is no further elaborate account of the Myxophyceæ from this Province. In this communication, fifty-nine forms of the Myxophyceæ have been recorded, and out of these three varieties and eight forms are new.

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<sup>1</sup> Berhampur is situated in latitude 24° 08' N. and longitude 88° 16' E., at a height of 67 feet above mean sea-level. The hottest months, namely, April and May, have a mean maximum temperature of 112.3° F., the highest temperature recorded being 115.1° F. The coldest months are December, January and February and they record a mean minimum temperature of 43.4° F., the extreme minimum temperature being 42.1° F. June, July, August and September are the chief rainy months and during this period the rainfall amounts to 42.96", the annual rainfall being 57.11".

<sup>2</sup> Puri is situated in latitude 99° 48' N. and longitude 85° 49' E., at a height of 24 feet above mean sea-level. The hottest months, namely, March and April, have a mean maximum temperature of 101.45° F., the highest temperature recorded being 102.2° F. The coldest months are December, January and February and they record a mean minimum temperature of 58.3° F., the extreme minimum temperature being 50.5° F. June to October are the chief rainy months, and during this period the rainfall amounts to 45.32" and the annual rainfall is about 54.56".

<sup>3</sup> Cuttack is situated in latitude 20° 29' N. and longitude 85° 52' E., at a height of 80 feet above mean sea-level. The hottest months, namely, May and June, have a mean maximum temperature of 115.7° F., the highest temperature being 117.7° F. The coldest months are December and January and they record a mean maximum temperature of 39° F., the extreme minimum temperature being 48.6° F. June to September are the chief rainy months, and during this period the rainfall amounts to 45.02", the annual rainfall being 59.27".

<sup>4</sup> In Khurda Road, June to September are the chief rainy months, and during this period the rainfall amounts to 43.91" and the annual rainfall is 59.29".

<sup>5</sup> Biswas, Kalipada—"Algal flora of the Chilka Lake," *Memoirs of the Asiatic Society of Bengal*, 1932, 11, No. 5, 165-198.

## SYSTEMATIC ENUMERATION OF THE SPECIES OBSERVED.

## I. CHROOCOCCALES.

*Chroococcaceæ.*Genus *Microcystis* Kützing.

1. *Microcystis æruginosa* Kuetz. Geitler in Rabenhorst's *Kryptogamenflora von Europa*, Band XIV, Cyanophyceæ, 1930-32, p. 136, Fig. 59 *d*; Frémy, "Les Myxophycées de Madagascar," *Annales de Cryptogamie exotique*, t. iii, fasc. IV, 1930, Pl. IV, Fig. 5; Crow, "The Taxonomy and variation of the genus *Microcystis* in Ceylon," *New Phytologist*, 1923, Vol. 22, No. 2, Pl. I, Fig. *a*; Tilden, *Minnesota Algæ*, Vol. I, 1910, Pl. II, Figs. 21 and 22.

Lat. cell., 3.5-5.2  $\mu$ .

*Habitat*:—In a pond, along with *Lyngbya majuscula* var. *chakiense* and others, Berhampur (4-11-36).

2. *Microcystis flos-aquæ* (Wittr.) Kirchn. Tilden, *op. cit.*, 1910, Pl. II, Fig. 18; Frémy, "Les Myxophycées de l'Afrique équatoriale française," *Arch. d. Bot.*, iii (1929), Mem. 2, 1930, p. 14, Fig. 16; Geitler, *op. cit.*, 1930-32, p. 136, Fig. 59 *e*.

Lat. cell., 4-6  $\mu$ .

*Habitat*:—In ponds and tanks, along with *Phormidium mucicola*, Cuttack (9-11-36).

Genus *Aphanocapsa* Nægeli.

3. *Aphanocapsa pulchra* (Kütz.) Rabenh. Geitler, *op. cit.*, 1930-32, p. 156, Fig. 69 *g*; Frémy, *op. cit.*, 1930, p. 23, Fig. 22.

Lat. cell., 3.2-4  $\mu$ .

*Habitat*:—On wet soil in a low-lying area, along with a sterile *Nostoc* sp., Puri (7-11-36).

Genus *Aphanothece* Nægeli.

4. *Aphanothece pallida* (Kütz.) Rabenh. Frémy, *op. cit.*, 1930, p. 33, Fig. 31.

Lat. cell., 5-7  $\mu$ ; long. cell., 7-11.8  $\mu$ .

*Habitat*:—In puddles by the side of the railway line, along with *Glæotrichia Raciborskii* var. *kashiense*, Puri (6-11-36); on wet soil in a rice-field, along with *Spirulina major*, Berhampur (5-11-36); on the pavement immersed in water in a tank, along with *Tolypothrix robusta* forma and others; in a pond among other algæ, Cuttack (8-11-36).

5. *Aphanothece bulbosa* (Menegh.) Rabenh. Frémy, *op. cit.*, 1930, p. 33, Fig. 33.

Lat. cell., 3.8-5.2  $\mu$ ; long. cell., 6-11  $\mu$ .

*Habitat* :—In a stagnant pond, along with *Aulosira Fritschii*, *Cosmarium* sp. and others, Berhampur (4–11–36).

6. *Aphanothece microscopica* Nag. Geitler, *op. cit.*, 1930–32, p. 173, Fig. 79; Tilden, *op. cit.*, 1910, Pl. II, Fig. 12.

Lat. cell., 4·5–5  $\mu$ ; long. cell., 8–9·5  $\mu$ .

*Habitat* :—In a rain-water puddle, along with other algæ, Berhampur (5–11–36).

#### Genus *Merismopedia* Meyen.

7. *Merismopedia tenuissima* Lemm. Geitler, *op. cit.*, 1930–32, p. 264, Fig. 129 b; Frémy, "Les Cyanophycées des Côtes d'Europe," *Memoires de la Société, Nationale des Sciences Naturelles et Mathématiques de Cherbourg*, tome XLI, 1934, Pl. 1, Fig. 1; Geitler, in Pascher's *Süsswasserflora Deutschlands, Osterreichs und der Schweiz.*, Heft 12, Cyanophyceæ, 1925, p. 107, Fig. 123 a.

Lat. cell., 1·2–2  $\mu$ .

*Habitat* :—In stagnant ponds, along with *Oscillatoria chalybea* and *Oscillatoria limosa*; in a waste-water drain, along with *Oscillatoria chalybea* and other algæ, Berhampur (5–11–36).

### II. CHÆMOSIPHONALES.

#### *Dermocarpaceæ*.

#### Genus *Stichosiphon* Geitler.

8. *Stichosiphon indica* Rao. Rao, "A new species of *Stichosiphon* (*Stichosiphon indica* sp. nov.)," *Proceedings of the Indian Academy of Sciences*, 1935, (B), Vol. 2, p. 536, Figs. 1–10.

Lat. sporang., 6–8  $\mu$ ; long. sporang., 70–150  $\mu$ ; lat. spor., 6–7·5  $\mu$ ; long. spor., 5–13  $\mu$ ; diam. spor., 5·5–7  $\mu$ ; crass. vag. upto 0·6  $\mu$ .

*Habitat* :—Epiphytic on *Cladophora* sp. growing on snails in rain-water pools, Berhampur (5–11–36).

### III. HORMOGONEALES.

#### 1. *Rivulariaceæ*.

#### Genus *Calothrix* Agardh.

9. *Calothrix fusca* Born. et Flah. Frémy, *op. cit.*, 1930, p. 246, Fig. 222.

*Forma* (Fig. 1, A and B).

Lat. fil., 9–14·8  $\mu$ , at top upto 4  $\mu$ ; long. fil., upto 350  $\mu$ ; lat. trich., 5·4–8·5 (–11·1)  $\mu$ , higher up 3–6  $\mu$  and at apex 0·7–1  $\mu$ ; long. cell., 2–8  $\mu$ ; lat. het., 5·4–8  $\mu$ ; long. het., 3·7–5·4 (–7·4)  $\mu$ ; crass. vag., upto 3  $\mu$ .

*Habitat* :—Adhering to a stone under water in a pond, Berhampur (5–11–36).

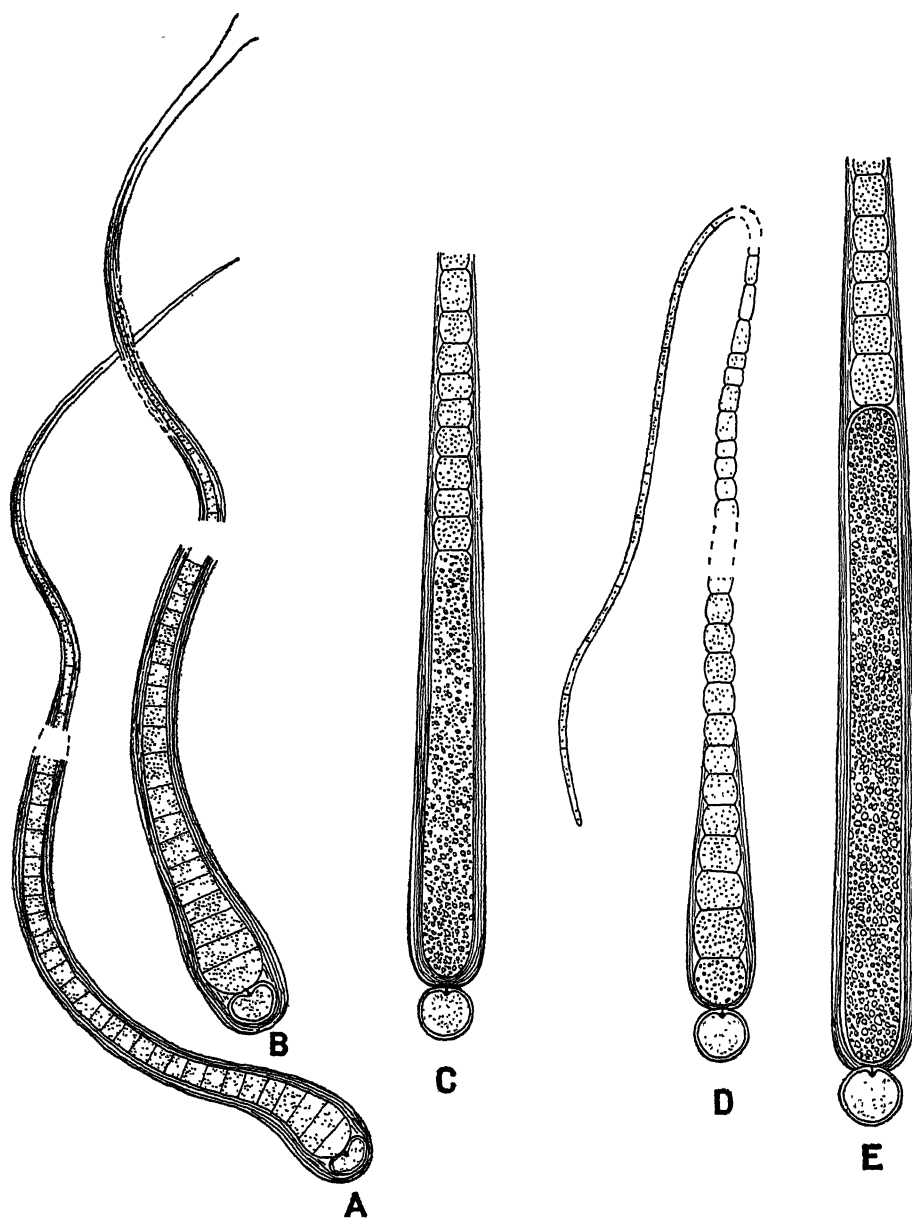


FIG. 1.—A and B—*Calothrix fusca* Born. et Flah. forma; C-E—Portions of filaments of *Glaettrichia echinulata* (J. E. Smith) P. Richter var. *berhampurensis* var. nov.

A and B  $\times 800$ ; C-E  $\times 660$ .

The form differs from the type in having closely entangled filaments, arranged in groups, and in the presence of the yellow-brown sheath.

Genus *Glæotrichia* Agardh.

10. *Glæotrichia Raciborskii* Woloszynska var. *kashiense* Rao. Rao, "The Myxophyceæ of the United Provinces, India—III," *Proceedings of the Indian Academy of Sciences*, 1937, (B), Vol. VI, No. 6, p. 352, Fig. 3, A–E.

Lat. trich., at base 8.4–10  $\mu$ , higher up 4.5–5.5  $\mu$  and at apex 2.8–3.5  $\mu$ ; long. trich., 800–1,000  $\mu$ ; long. cell., at base 3.5–5  $\mu$ , later 3.5–13.2  $\mu$ , in the hair upto 15  $\mu$  and at apex upto 20  $\mu$ ; lat. het., 8–13.2  $\mu$ ; long. het., 8.2–13.2 (–16)  $\mu$ ; lat. spor., 11.5–14  $\mu$ , long. spor., 62–69  $\mu$ .

*Habitat* :—In a puddle by the side of the railway line, along with *Aphanothece pallida*, Puri (7–11–36); in a puddle, Cuttack (9–11–36).

11. *Glæotrichia echinulata* (J. E. Smith) P. Richter. Geitler, *op. cit.*, 1930–32, p. 642, Fig. 409; Tilden, *op. cit.*, 1910, Pl. 20, Fig. 4.

Var. *berhampurensis* var. nov. (Fig. 1, C–E).

Thallus forming small, spherical, hard masses, 1–2 mm. in diameter. Filaments with a firm, thick, stratified, yellow-brown sheath. Trichomes with constrictions at the joints and ending in a hair. Cells at the base of the trichome barrel-shaped, usually longer than broad, higher up quadratic or cylindrical, in the hair long cylindrical. Heterocysts, single, spherical or sub-spherical. Spores cylindrical with a smooth hyaline outer wall.

Lat. trich., at base 6–9  $\mu$ , higher up 5–6  $\mu$  and at apex 2–4  $\mu$ ; long. trich., upto 400  $\mu$ ; long. cell at base 4–10  $\mu$ , higher up 4–8  $\mu$ , at apex 16–18  $\mu$ ; lat. het., 9–12.2  $\mu$ ; long. het., 9.8–12.2  $\mu$ ; lat. spor., 7.8–9.5  $\mu$ ; long. spor., 67–205  $\mu$ ; lat. spor. cum mem., 11.7–15  $\mu$ ; crass. vag., upto 3  $\mu$  and rarely upto 5  $\mu$ .

*Habitat* :—Adhering to *Chara* sp. and other aquatic angiosperms in a pond, Berhampur (5–11–36).

The variety agrees with the type in all respects except that the former has a firm, thick, stratified, yellow-brown sheath and much longer and comparatively narrower spores.

2. *Microchætaceæ*.

Genus *Aulosira* Kirchner.

12. *Aulosira fertilissima* Ghose var. *tenuis* Rao. Rao, *op. cit.*, 1937, p. 352, Fig. 3, F–I.

Lat. fil., 5–6.2  $\mu$ ; crass. vag., 0.3–0.8  $\mu$ ; lat. trich., 3.3–4.8  $\mu$ ; long. cell., 4–13.2  $\mu$ ; lat. het., 4.5–6.6  $\mu$ ; long. het., 8.2–18.1 (–23.2)  $\mu$ ; lat. spor., 4.8–8.2  $\mu$ ; long. spor., 10–18.8  $\mu$ .

*Habitat* :—In several stagnant ponds, along with *Tolypothrix robusta* forma, Berhampur (4–11–36).

13. *Aulosira Fritschii* Bhâradwâja. Bhâradwâja, "Contributions to our Knowledge of the Myxophyceæ of India," *Annals of Botany*, 1933, Vol. 47, 185, pp. 123-131, Figs. 3 and 4.

Lat. fil., 10-16.5  $\mu$ ; crass. vag., upto 2.8  $\mu$ ; lat. trich., 8-10  $\mu$ ; long. cell., 8-23.2  $\mu$ ; lat. het., 8.2-13.2  $\mu$ ; long. het., 16.5-19.8  $\mu$ ; lat. spor., 10-13.2  $\mu$ ; long. spor., 6-23.2  $\mu$ .

*Habitat*:—In a pond, along with *Nostoc* sp., *Oedogonium* sp., *Pediastrum* sp. and others, Cuttack (8-11-36); in a puddle by the side of the railway line, along with *Anabaena Iyengari* var. *attenuata*, *Pithophora* sp., *Lyngbya confervoides* and others, Cuttack (9-11-36); in a rain water pool, along with *Tolypothrix robusta* and *Cylindrospermum muscicola*, Puri (6-11-36).

### 3. *Scytonemataceæ*.

#### Genus *Tolypothrix* Kützing.

14. *Tolypothrix robusta* Gardner forma Rao. Rao, *op. cit.*, 1937, p. 354. Diam. fil., 13.2-16.6  $\mu$ , when old upto 20  $\mu$ ; crass. vag., 2-3.5  $\mu$ , when old and unhealthy upto 5.5  $\mu$ ; lat. trich., 10-12.5  $\mu$ , when old and unhealthy narrowed down to 5  $\mu$ , at growing apices upto 14.4  $\mu$ ; long. cell., 8-15  $\mu$ , when old and unhealthy 26-36  $\mu$ , at growing apices 4-6  $\mu$ ; lat. het., 10-13  $\mu$ ; long. het., 13-40  $\mu$ .

*Habitat*:—In a pond, along with sterile *Cylindrospermum* sp., Puri (7-11-36); in an irrigation channel; in stagnant ponds, Berhampur (5-11-36); in a stagnant pond, along with *Aulosira fertilissima* var. *tenuis*, Berhampur (4-11-36); in a rain-water pool, along with *Aulosira Fritschii*, *Cylindrospermum muscicola*, and others, Puri (7-11-36).

#### Genus *Scytonema* Agardh.

15. *Scytonema ocellatum* Lyngbye. Frémy, *op. cit.*, 1930, p. 309, Fig. 263.

Lat. fil., 16-19.5  $\mu$ , when old upto 21  $\mu$ ; crass. vag., 2-4  $\mu$ , when old and unhealthy upto 7  $\mu$ ; lat. trich., 11-14  $\mu$ , when old and unhealthy narrowed down to 8  $\mu$ ; long. cell., 6-14.5  $\mu$ , when old and unhealthy upto 19  $\mu$ ; lat. het., 12-14  $\mu$ ; long. het., 13-16  $\mu$ .

*Habitat*:—On a wall in shade, Puri (7-11-36).

### 4. *Nostocaceæ*.

#### Genus *Cylindrospermum* Kütz.

16. *Cylindrospermum muscicola* Kütz. Frémy, *op. cit.*, 1930, p. 377, Fig. 313; Tilden, *op. cit.*, 1910, Pl. X, Fig. 6; Ghose, "On some Myxophyceæ from Rangoon," *Journal of the Burma Research Society*, Vol. XV, Part III, pp. 244-253, Pl. VII, Fig. 15.

Lat. cell.,  $2.8-3\ \mu$ ; long. cell.,  $2.8-5\ \mu$ ; lat. het.,  $4-4.8\ \mu$ ; long. het.,  $5-7.8\ \mu$ ; lat. spor.,  $9-10\ \mu$ ; long. spor.,  $16-21.5\ \mu$ .

*Habitat*:—In a rain-water pool, along with *Tolypothrix robusta* forma, *Aulosira Fritschii* and others, Puri (6-11-36).

Genus *Anabæna* Bory.

17. *Anabæna Iyengari* Bhâradwâja. Bhâradwâja, "The Myxophyceæ of the United Provinces, India—I," *Proceedings of the Indian Academy of Sciences*, 1935, (B), Vol. II, 1, p. 106, Fig. 6, H-K.

Var. *attenuata* var. nov. (Fig. 2, A-C).

Plant-mass mucilaginous, deep blue-green. Trichomes single, straight or irregularly curved; tapering at the ends; end-cells conical with rounded apices. Cells barrel-shaped, as long as broad or shorter or longer than broad. Heterocysts barrel-shaped. Spores ellipsoidal, single or in pairs on either side of a heterocyst, with a smooth hyaline outer wall.

Lat. cell.,  $4-4.2\ \mu$ , at apex  $1.5-2\ \mu$ ; long. cell.,  $3-6.4\ \mu$ ; lat. het.,  $5-6\ \mu$ ; long. het.,  $6-9\ \mu$ ; lat. spor.,  $11.7-15.6\ \mu$ ; long. spor.,  $15.4-19.5\ \mu$ .

*Habitat*:—In a puddle by the side of the railway line, along with *Pithophora* sp., *Aulosira Fritschii*, *Lyngbya confervoides* and other algæ, Cuttack (8-11-36).

The variety resembles the type in the barrel-shaped cells, conical end-cells, with rounded apices, barrel-shaped heterocysts and ellipsoidal spores, that are on either side of a heterocyst; but differs from the same in having narrower trichomes with tapering ends, narrower heterocysts and broader spores with hyaline outer wall and situated singly or in pairs on either side of a heterocyst.

5. *Oscillatoriaceæ*.

Genus *Spirulina* Turpin.

18. *Spirulina subsalsa* Oerst. Geitler, *op. cit.*, 1930-32, p. 928, Fig. 593 a; Tilden, *op. cit.*, 1910, Pl. IV, Fig. 49.

Lat. trich.,  $1.5-2.2\ \mu$ ; lat. spir.,  $3-5\ \mu$ .

*Habitat*:—Among other algæ, in several drains; in a rice-mill tank, along with *Oscillatoria princeps*, *O. chalybea* and others, Berhampur (4-11-36).

19. *Spirulina major* Kütz. Geitler, *op. cit.*, 1930-32, p. 930, Fig. 595; Frémy, *op. cit.*, 1930, p. 235, Fig. 208; Tilden, *op. cit.*, 1910, Pl. IV, Fig. 46; Frémy, *op. cit.*, 1934, Pl. XXXI, Fig. 18; Carter, "A comparative study of the Algal flora of two Salt Marshes, Part II," *Journal of Ecology*, Vol. XXI, I, 1933, p. 159, Fig. 2; Ghose, *op. cit.*, 1926, Pl. VI, Fig. 3.

Lat. trich.,  $1-1.4\ \mu$ ; lat. spir.,  $2.4-3.5\ \mu$ ; spat. inter duo. spir.,  $2.4-3.2\ \mu$ .

*Habitat* :—On wet soil in a rice-field along with *Aphanothece pallida* and others ; in many waste-water drains, along with other algæ, Berhampur (5-11-36).

Genus *Oscillatoria* Vauch.

20. *Oscillatoria nigro-viridis* Thwaites. Tilden, *op. cit.*, 1910, Pl. IV, Fig. 12.

Lat. cell.,  $7.2-9.6\ \mu$  ; long. cell.,  $2.4-4\ \mu$ .

*Habitat* :—In a rain-water puddle, along with *Oscillatoria sancta* and others, Puri (11-11-36).

21. *Oscillatoria sancta* (Kütz.) Gom. Frémy, *op. cit.*, 1930, p. 210, Fig. 177 ; Tilden, *op. cit.*, 1910, Pl. IV, Fig. 5 ; Carter, *op. cit.*, 1933, p. 159, Figs. 11 and 12 ; Geitler, *op. cit.*, 1925, p. 356, Fig. 418.

Lat. trich.,  $10-12\ \mu$  ; long. cell.,  $2-3\ \mu$ .

*Habitat* :—On a moist brick pavement near a waste-water drain, along with other algæ, Cuttack (8-11-36).

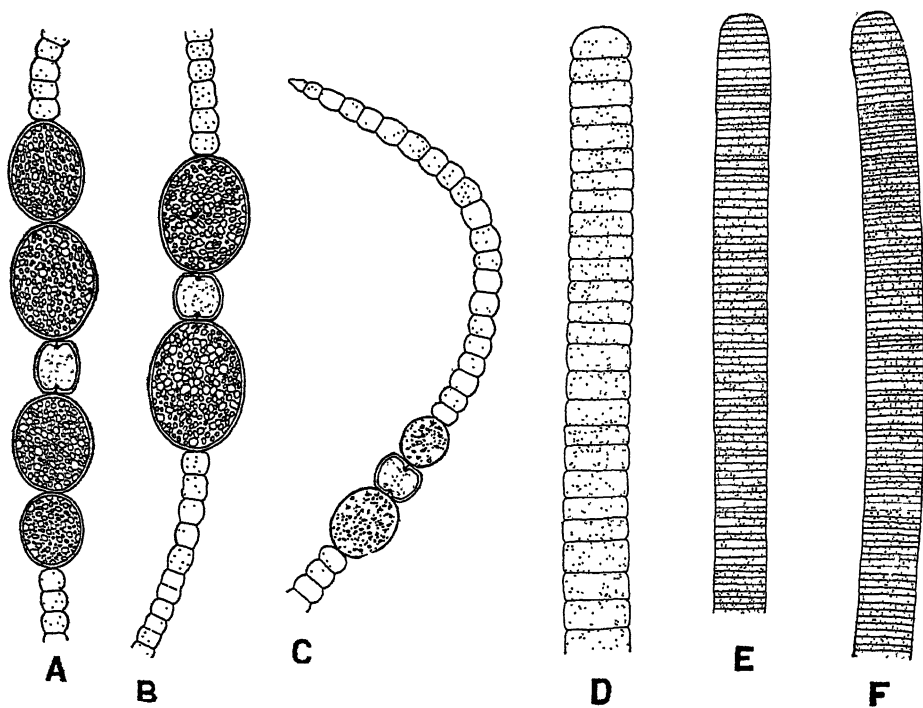


FIG. 2.—A-C—*Anabaena Iyengari* Bhāradwāja var. *attenuata* var. nov.; D—Terminal portion of *Oscillatoria ornata* Kütz var. *crassa* var. nov.; E and F—Terminal portions of *Oscillatoria curviceps* Ag. forma.

A-C  $\times 800$  ; D  $\times 660$  ; E and F  $\times 400$ .

22. *Oscillatoria ornata* Kütz. Tilden, *op. cit.*, 1910, Pl. IV, Fig. 8.

Var. *crassa* var. nov. (Fig. 2 D).

Plant-mass dark blue-green. Trichomes straight and of uniform thickness; with constrictions at the joints; septa granulated. Cells shorter than broad; end-cell convex without cap or calyptra.

Lat. trich., 11–15  $\mu$ ; long. cell., 2–5.5  $\mu$ .

*Habitat*:—In a pond, along with *Oscillatoria chalybea*, *P. curviceps* forma and sterile *Spirogyra* sp., Cuttack (9–11–36).

This variety differs from the type in having broader trichomes with straight apices.

23. *Oscillatoria limosa* Ag. Geitler, *op. cit.*, 1930–32, p. 943, Fig. 598 d.

Lat. trich., 10.8–12  $\mu$ ; long. cell., 2.5–3.8  $\mu$ .

*Habitat*:—In a stagnant pond, along with *Oscillatoria chalybea* and *Merismopedia tenuissima*, Berhampur (5–11–36).

24. *Oscillatoria obscura* Brühl and Biswas. Brühl and Biswas, "Algæ of the Bengal Filter Beds," *Journal of the Department of Science*, Calcutta University, Vol. IV, 1922, Pl. II, Fig. 9.

Lat. cell., 3.7–4  $\mu$ ; long. cell., 1–1.2  $\mu$ .

*Habitat*:—In a pond, along with *Oscillatoria anguina*, *O. articulata* and other algæ, Berhampur (4–11–36).

25. *Oscillatoria princeps* Vauch. Geitler, *op. cit.*, 1930–32, p. 943, Fig. 598 a, and p. 946, Fig. 601 c–g.

Lat. trich., 24–35  $\mu$ ; long. cell., 4–6  $\mu$ .

*Habitat*:—In ponds singly or along with *Oedogonium* sp. and several others; in a rice mill tank, along with *Spirulina subsalsa*, *Oscillatoria chalybea* and others; in a drain, along with *Spirulina major* and other algæ, Berhampur (5–11–36).

26. *Oscillatoria curviceps* Ag. Tilden, *op. cit.*, 1910, Pl. IV, Fig. 7.

*Forma* (Fig. 2 E and F).

Lat. trich., 14–17.8  $\mu$ ; long. cell., 2–4  $\mu$ .

*Habitat*:—In a pond, along with *Oscillatoria chalybea*, *O. ornata* var. *crassa* and sterile *Spirogyra* sp., Cuttack (9–11–36).

The form agrees with the type in all respects except that the former possesses trichomes with straight apices.

27. *Oscillatoria anguina* (Bory) Gom. Geitler, *op. cit.*, 1930–32, p. 945, Fig. 599 b.

Lat. trich., 6–6.8  $\mu$ ; long. cell., 1.6–3  $\mu$ .

*Habitat* :—In a pond, along with *Oscillatoria obscura*, *O. articulata* and others, Berhampur (4-11-36).

28. *Oscillatoria Martini* Frémy. Frémy, *op. cit.*, 1930, p. 229, Fig. 203, *Forma*.

Lat. trich., 4.5-5  $\mu$ ; long. cell., 2-4  $\mu$ .

*Habitat* :—In a pond, along with other algæ, Berhampur (5-11-36).

But for the narrower trichomes, the form agrees with the type in all respects.

29. *Oscillatoria terebriformis* Ag. Geitler, *op. cit.*, 1930-32, p. 955, Fig. 607 d.

Lat. trich., 4-4.5  $\mu$ ; long. cell., 2-4  $\mu$ .

*Habitat* :—In a waste-water drain, along with *Oscillatoria Boryana*, Khurda Road (7-11-36).

*Forma* Rao. Rao, *op. cit.*, 1936, p. 171.

Lat. trich. 4-4.2  $\mu$ , long. cell., 3-4.5  $\mu$ .

*Habitat* :—On stones near a house outlet, along with *Oscillatoria variabilis*, Puri (6-11-36).

30. *Oscillatoria Boryana* Bory. Geitler, *op. cit.*, 1930-32, p. 955, Fig. 607 b and c.

Lat. trich., 5-7  $\mu$ , at apex 3  $\mu$ ; long. cell., 3-5  $\mu$ .

*Habitat* :—In a waste-water drain, along with *Oscillatoria terebriformis*, Khurda Road (7-11-36); in a drain, along with others, Berhampur (4-11-36).

31. *Oscillatoria chalybea* Mertens. Geitler, *op. cit.*, 1930-32, p. 956, Fig. 608 b.

Lat. cell., 9-12.8  $\mu$ ; long. cell., 3.5-7  $\mu$ .

*Habitat* :—In stagnant ponds, along with *Merismopedia tenuissima* and *Oscillatoria limosa*; in a rice-mill tank, along with *Oscillatoria princeps*, *Spirulina subsalsa* and others, Berhampur (5-11-36); in a waste-water drain, along with *Oscillatoria animalis*; in a puddle, along with *Merismopedia tenuissima* and others, Puri (7-11-36).

32. *Oscillatoria tenuis* Ag. Tilden, *op. cit.*, 1910, Pl. IV, Fig. 17.

Lat. cell., 6-7  $\mu$ ; long. cell., 2-3  $\mu$ .

*Habitat* :—In a rain-water puddle, Berhampur (4-11-36).

33. *Oscillatoria articulata* Gardner. Geitler, *op. cit.*, 1930-32, p. 963, Fig. 614 a.

Lat. trich.,  $3-3.5\ \mu$ ; long. cell.,  $2-3.7\ \mu$ .

*Habitat* :—In a pond, along with *Oscillatoria anguina*, *O. obscura* and others, Berhampur (5-11-36).

34. *Oscillatoria pseudogeminata* G. Schmid. Geitler, *op. cit.*, 1930-32, p. 966, Fig. 616.

Lat. trich.,  $1.8-2\ \mu$ ; long. cell.,  $1.8-2.9\ \mu$ .

*Habitat* :—On stones near a house outlet, along with other algæ, Cuttack (8-11-36).

35. *Oscillatoria homogenea* Frémy. Frémy, *op. cit.*, 1930, p. 221, Fig. 184.

Lat. trich.,  $3.4-3.6\ \mu$ ; long. cell.,  $4-6\ \mu$ .

*Habitat* :—In a pond, along with sterile *Spirogyra* sp., Berhampur (5-11-36).

36. *Oscillatoria Okeni* Ag. Geitler, *op. cit.*, 1930-32, p. 956, Fig. 608 a.

Lat. trich.,  $4.9-6\ \mu$ ; long. cell.,  $2.5-5\ \mu$ .

*Habitat* :—In a pond, along with *Microcystis æruginosa*, Berhampur (5-11-36); in a sewage drain opening into the sea, Puri (7-11-36).

37. *Oscillatoria formosa* Bory. Geitler, *op. cit.*, p. 971, Fig. 619 b.

Lat. trich.,  $4.5-6\ \mu$ ; long. cell.,  $2-4.5\ \mu$ .

*Habitat* :—In several waste-water drains, Cuttack (8-11-36).

38. *Oscillatoria animalis* Ag. Geitler, *op. cit.*, 1930-32, p. 950, Fig. 603 e.

Lat. trich.,  $3-4\ \mu$ , near apex  $2\ \mu$ ; long. cell.,  $2-5\ \mu$ .

*Habitat* :—In a waste-water drain, along with *Oscillatoria chalybea*, Puri (6-11-36).

39. *Oscillatoria variabilis* Rao. Rao, "The Myxophyceæ of the United Provinces, India—II," *Proceedings of the Indian Academy of Sciences*, (B), 1936, III, 2, p. 172, Fig. 3, A-D.

Lat. trich.,  $5.2-5.5\ \mu$ ; long. cell.,  $3-4.8\ \mu$ .

*Habitat* :—On stones near a house outlet, along with *Oscillatoria terebriiformis* forma, Puri (6-11-36).

*Forma.*

Lat. trich.,  $5-5.5\ \mu$ ; long. cell.,  $2.8-4.5\ \mu$ .

*Habitat* :—On moist soil near a water pipe, Berhampur (5-11-36).

The form differs from the type in the trichomes usually tapering at the extreme apices and possessing end-cells with their outer walls thickened.

Genus *Phormidium* Kütz.

40. *Phormidium muscicola* Hub.—Pestalozzi et Naum. Geitler, *op. cit.*, 1930–32, p. 998, Fig. 637.

Lat. cell.,  $1.4-1.5\ \mu$ ; long. cell.,  $1.3-1.8\ \mu$ .

*Habitat*:—In ponds and tanks, along with *Microcystis flos-aquæ*, Cuttack (4–11–36).

41. *Phormidium Bohneri* Schmidle forma Rao. Rao, *op. cit.*, 1936, p. 173.

Lat. trich.,  $1.7-2\ \mu$ ; long. cell.,  $1.3-2.2\ \mu$ .

*Habitat*:—On an iron water pipe; in a cemented waste-water drain, Berhampur (5–11–36).

42. *Phormidium cebennense* Gom. Frémy, *op. cit.*, 1930, p. 147, Fig. 129.

Lat. trich.,  $1.8-2.2\ \mu$ ; long. cell.,  $0.8-2\ \mu$ .

*Habitat*:—On the sides of a stone platform in a tank (Chandan Talav), Puri (6–11–36); on the steps of a tank; on stones near a well, Cuttack (8–11–36) and (9–11–36); on bricks near a water tap, along with *Phormidium anomala*, Berhampur (1–1–37).

43. *Phormidium purpurascens* (Kütz.) Gom. Tilden, *op. cit.*, 1910, Pl. IV, Fig. 59.

Lat. fil.,  $3-4\ \mu$ ; crass. vag.,  $0.5\ \mu$ ; lat. trich.,  $2-2.5 (-2.8)\ \mu$ ; long. cell.,  $2-5\ \mu$ .

*Habitat*:—In a cemented water course near a water-tap, Cuttack (9–11–36); on stones in a pond, Berhampur (Rao, J. Varahagiri, 1–1–37).

44. *Phormidium Retzii* (Ag.) Gom. Geitler, *op. cit.*, 1930–32, p. 1012, Fig. 647 a–d.

Lat. fil.,  $5-7.5\ \mu$ ; lat. trich.,  $4.5-7.2\ \mu$ ; long. cell.,  $4.2-7.4\ \mu$ .

*Habitat*:—Adhering to water plants in a pond, Cuttack (8–11–36); among aquatic angiosperms in ponds, Berhampur (5–11–36).

45. *Phormidium pachydermaticum* Frémy. Frémy, *op. cit.*, 1930, p. 159, Fig. 138.

*Forma.*

Lat. trich.,  $3.2-4.2\ \mu$ ; long. cell.,  $4-8\ \mu$ .

*Habitat*:—On the wet steps of a tank, Berhampur (4–11–36).

The form differs from the type in the trichomes being narrower and the cells being usually longer than broad.

46. *Phormidium Corium* Gom. Tilden, *op. cit.*, 1910, Pl. IV, Figs. 71 and 72.

Lat. trich.,  $3-3.5\ \mu$ ; long. cell.,  $3.5-6.5\ \mu$ .

*Habitat* :—On cemented surface near a water tap, Cuttack (8-11-36).

Var. *capitatum* Gardner. Geitler, *op. cit.*, 1930-32, p. 1017, Fig. 649 d.

Lat. trich.,  $4.8-5.4\ \mu$ ; long. cell.,  $3.8-6.8\ \mu$ .

*Habitat* :—On stones in a channel, Berhampur (5-11-36).

47. *Phormidium papyraceum* Gom. Tilden, *op. cit.*, 1910, Pl. IV, Figs. 73 and 74.

Lat. trich.,  $4-5\ \mu$ ; long. cell., (2-)  $4.2-5\ \mu$ .

*Habitat* :—On the bark of a tree, Puri (7-11-36).

48. *Phormidium favosum* (Bory) Gom. Tilden, *op. cit.*, 1910, Pl. V, Figs. 9 and 10.

Lat. trich.,  $4-6\ \mu$ ; long. cell.,  $3-6\ \mu$ .

*Habitat* :—On mud in a drain, Cuttack (8-11-36).

49. *Phormidium autumnale* (Ag.) Gom. Tilden, *op. cit.*, 1910, Pl. V, Figs. 18 and 19.

*Forma*.

Lat. trich.,  $4-5\ \mu$ , at apex  $4\ \mu$ ; long. cell.,  $1-2\ \mu$ .

*Habitat* :—On a moist pavement near a water tap, Khurda Road (7-11-36).

But for the shorter cells, this form agrees with the type in all respects.

50. *Phormidium anomala* Rao. Rao, *op. cit.*, 1937, p. 370, Fig. 7, F-I.

Lat. trich.,  $9.8-10.5\ \mu$ ; long. cell.,  $1-1.8\ \mu$ .

*Habitat* :—On bricks near a water tap, Berhampur (2-1-37).

#### Genus *Lyngbya* Agardh.

51. *Lyngbya rubida* Frémy. Frémy, *op. cit.*, 1930, 3, 185, Fig. 155.

*Forma*.

Lat. fil.,  $7.5-8.5\ \mu$ ; lat. trich.,  $5.5-6.6\ \mu$ ; long. cell.,  $5-9\ \mu$ ; crass. vag., upto  $1\ \mu$ .

*Habitat* :—In a stagnant pond, Berhampur (5-11-36).

The form differs from the type in having broader trichomes with a hyaline sheath and shorter cells with minute constrictions near the septa.

52. *Lyngbya majuscula* Harv. var. *chakiense* Rao. Rao, *op. cit.*, 1936, p. 172, Figs. 3, E and F.

Lat. fil.,  $36-56\ \mu$ ; lat. trich.,  $26-33\ \mu$ ; long. cell.,  $3.5-6\ \mu$ ; crass. vag.,  $5-6.6\ (-10)\ \mu$ .

*Habitat* :—In a pond, along with *Microcystis æruginosa* and others; in channels; in ponds among other water plants, Berhampur (4-11-36 and 5-11-36).

53. *Lyngbya confervoides* Ag. Tilden, *op. cit.*, 1910, Pl. V, Fig. 39; Frémy, *op. cit.*, 1934, Pl. 28, Fig. 2; Carter, *op. cit.*, 1933, p. 162, Fig. 11, 1 and 2.

Lat. fil., 18–19.5  $\mu$ ; lat. trich., 13–16  $\mu$ ; long. cell., 2–3  $\mu$ ; crass. vag., upto 4  $\mu$ .

*Habitat*:—In a puddle by the side of the railway line, along with *Anabaena Iyengari* var. *attenuata*, *Aulosira Fritschii*, *Pithophora* sp. and other algæ, Cuttack (8–11–36).

54. *Lyngbya ærugineo-cærulea* (Kütz.) Gom. Frémy, *op. cit.*, 1930, p. 193, Fig. 157.

Lat. fil., 6.6–8.2  $\mu$ ; lat. trich., 4–6  $\mu$ ; long. cell., 1.5–5.2  $\mu$ ; crass. vag., upto 1.6  $\mu$ .

*Habitat*:—On the cemented platform near a water tap, Cuttack (8–11–36); on the steps in a tank, Berhampur (5–11–36).

55. *Lyngbya putealis* Mont. Frémy, *op. cit.*, 1930, p. 193, Fig. 159, *a* and *b*; Tilden, *op. cit.*, 1910, Pl. V, Fig. 45.

Lat. fil., 7–10  $\mu$ ; lat. trich., 5–8.5  $\mu$ ; long. cell., 3–10  $\mu$ ; crass. vag., 1  $\mu$ .

*Habitat*:—On the steps of a tank, Berhampur (5–11–36).

#### Genus *Symplocà* Kuetzing.

56. *Symplocà muralis* Kütz. Geitler, *op. cit.*, 1930–32, p. 1125, Fig. 732; Frémy, *op. cit.*, 1930, p. 129, Fig. 113 *a* and *b*; West, *Algæ*, I, 1916, p. 23, Fig. 15 *E*.

Lat. fil., 5–5.5  $\mu$ ; lat. trich., 4–4.5  $\mu$ ; long. cell., 3–4  $\mu$ .

*Habitat*:—On moist soil near a pond, Berhampur (4–11–36).

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# THE CYTOLOGY OF DIGESTION AND ABSORPTION IN THE CRAB *PARATELPHUSA* (*OZIOTELPHUSA*) *HYDRODROMUS* (HERBST).

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## *Introduction.*

IN a previous communication, an account of some investigations into the physiology of digestion and absorption in *Paratelphusa* (*Oziotelphusa*) *hydromomus* (Herbst) has been given (Reddy, 1937). The present paper deals with the cytology of the epithelial cells of the digestive tubules, midgut and its cæca. The author is not aware of any published paper which deals with the distribution and behaviour of the cytoplasmic inclusions within the epithelial cells during the process of digestion and absorption in *Paratelphusa* (*Oziotelphusa*) *hydromomus* (Herbst).

## *Material and Method.*

The crabs were killed after varying periods of starvation and different intervals of feeding and material was fixed in all cases in Bouin, Gilson and Flemming. In the case of the digestive gland 30 per cent. alcohol containing 5 per cent. corrosive sublimate was also used as a fixative. Animals fed on olive oil stained with Sudan III were fixed in Fleming without acetic acid, while animals fed on ferrum oxydatum saccharatum, were fixed in 95 per cent. alcohol containing 5 per cent. ammonium sulphide and the presence of iron in the absorptive cells was shown by the Prussian Blue reaction.

Living material was stained with neutral red in order to make out the origin of the secretory granules in the cells. The subsequent transformation into fully formed secretory granules was also followed.

Mann Kopsch method was found to be most satisfactory in the study of the Golgi apparatus while Flemming without acetic acid gave the best results for mitochondria.

## *Observations.*

(1) *Digestive tubules.*—The digestive gland consisting of a right and a left half occupies the entire anterior region of the cephalothorax. Each half comprises a large number of blindly ending tubules. The lumina of these tubules are continuous with three main ducts corresponding to the

three main lobes. The three main ducts on each side join together and open into the midgut.

In a transverse section each digestive tubule is bound externally by connective tissue internal to which is the basement membrane, next to which is the lining layer of columnar epithelial cells. At the base of the epithelial cells are small cells, the basal cells with large centrally situated spherical or oval nuclei. The epithelial cells are of two types, namely: (i) the secretory cells and (ii) the absorptive cells. These two types of cells occur side by side and are not confined to any well-defined region.

(i) *Secretory cells*.—The old functionally inactive secretory cells are  $70\ \mu$  in length and  $60\ \mu$  in width. The very young secretory cells are about  $20\ \mu$  in length. Each cell is provided with a small round nucleus towards the base of the cell. Towards the lumen each cell has a striated border which is very often distorted by the action of the fixing agents thus producing a ciliated appearance. An examination of stained living material clearly shows the absence of cilia.

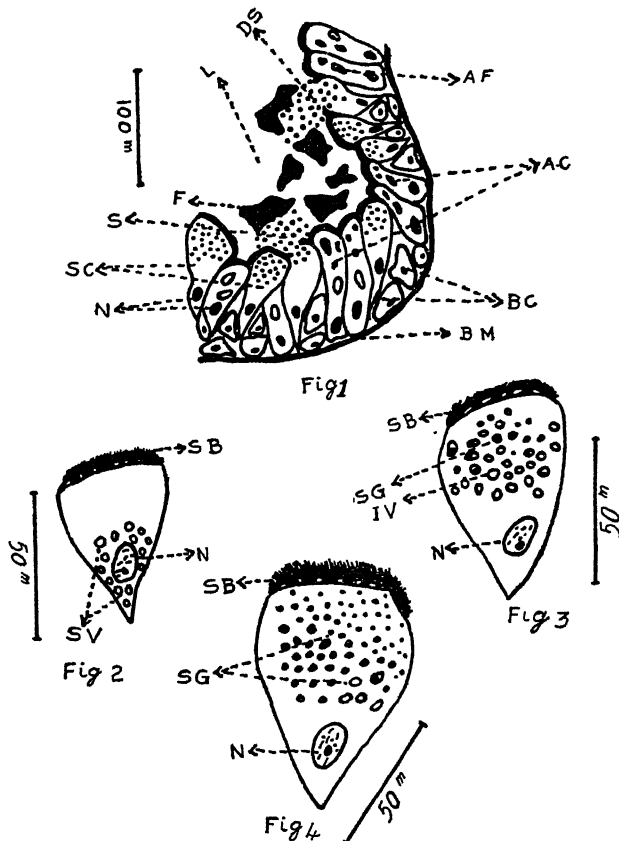
In many of the secretory cells the portion next to the lumen, the zymogen region projects into the lumen in the form of a dilatation. It is packed with secretory granules. In some sections these cells are found discharging their contents into the lumen by the rupture of their striated borders. In some other sections old secretory cells devoid of secretory granules are found. These cells have evidently repaired their ruptured striated borders after discharging their secretions. Some of the cells detach *en masse* their sac-like dilatations containing secretion into the lumen. These masses of granules stain darkly in sections while the ruptured free ends of the secretory cells are faintly stained in the sections.

In two or three cases the secretory cells were found to be degenerating. The degeneration probably sets in after a long period of activity. The basal cells presumably get differentiated into the secretory cells and replace the degenerated secretory cells as pointed out by Yonge (1924) in *Nephrops norvegicus*. In some sections there are a few cells of varying lengths. They probably represent intermediate stages of growth from basal cells to young secretory cells. The mode of replacement of cells in the midgut in *Periplaneta* (Gresson, 1934) recalls the condition in the material under study. In the base of the secretory cells no trace of secretory granules could be detected. In some which are not very much different from young secretory cells formation of secretory granules was noticed as revealed by neutral red staining.

Fresh material from the digestive tubules was stained with neutral red. In some of the young secretory cells small vacuoles which were stained by

neutral red were found. These were found towards the basal region of the cell around the nucleus in the prezymogen region. In the old cells, neutral red staining vacuoles along with large granules which were also stained by neutral red were found towards the striated border, within the zymogen region. Some of the neutral red staining vacuoles were partially or totally stained in their interior also. In a few of the old cells, only large granules which were stained by neutral red along with a large number of very small granules unstained by neutral red were found towards the region next to the lumen. In some very large secretory cells which protruded into the lumen the granules which were packed in the protruded portion, did not take the neutral red stain at all. The granules are very small when compared to the large granules in some cells. In the latter case, the granules were stained by neutral red.

All the above observations indicate that the early secretion arises in small vacuoles in the prezymogen region at the basal portion. Then it moves



*N.B.*—In the scale by the side of each text-figure read “μ” instead of “m”.

into the zymogen region where the vacuoles get converted into granules by the secretory material being formed in them. Later, these granules decrease in size and constitute mature secretory granules which are ready to be discharged into the lumen.

(ii) *Absorption cells*.—The absorption cells are vacuolate and vary from  $70\ \mu$  to  $110\ \mu$  length. Their nuclei are oval or round and are found towards the basal region. Their free borders are striated. These extend further into the lumen and do not show the presence of any secretory granules. In some preparations are found large granules clumped together. These are particularly manifest in Mann Kopsch and Flemming preparations. These granules are the fatty food materials absorbed by these cells. The absorptive nature of these cells is confirmed by the Prussian Blue reaction in the case of animals fed on iron salts. The absorbed food material is enclosed within vacuoles. No material was absorbed by the secretory or the basement cells. In some of the absorption cells stored fats were found. These were used up during continued periods of starvation. The absorption cells in such cases were all shrunk and distorted.

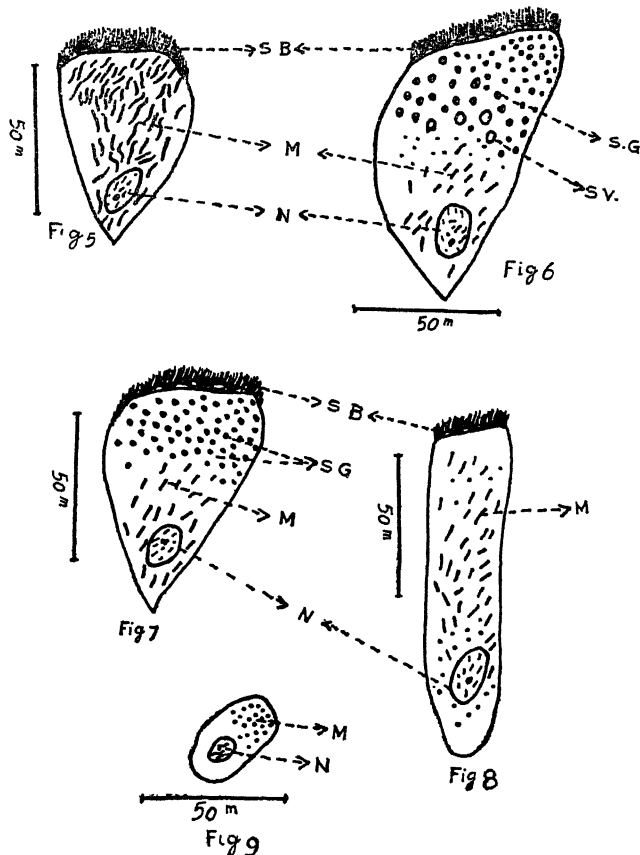
(2) *Midgut and its cæca*.—The lining epithelium of the midgut and its cæca consists of columnar cells which are  $50\ \mu$  in length with oval nuclei towards their bases. Their striated border is about 1 to  $2\ \mu$  in thickness. Near the base of the epithelium, the basal cells are present. All the epithelial cells of the midgut and its cæca are absorptive in function. Spheres of food material absorbed from the lumen were enclosed in the vacuoles present in these cells. As in the case of the basal cells of the digestive tubules, no absorbed material or secretory granules are found in the basal cells of the midgut and its cæca.

(3) *Cytoplasmic inclusions*.—(a) *Mitochondria*. In young secretory cells numerous filamentous mitochondria are found throughout the cell. These mitochondria are clumped together towards the border of the cell next to the lumen (Fig. 5). Large number of rod-like and granular mitochondria are found towards the basal region and middle portion of the older secretory cells which are packed with formed secretory granules (Fig. 6). But here the mitochondria towards the basal portion are rod-like and are not so packed as in the younger cells. In some cells a few granular mitochondria are also found towards the region next to the lumen. But in the majority of cells mitochondria are not found in this region.

In the case of secretory cells which have discharged their secretion into the lumen and which are to enter another phase of secretory activity, the mitochondria are somewhat like those of young secretory cells. They are

filamentous around the nucleus and in the middle region of the cell. But they are, however, granular towards the striated border.

In the absorption cells of the midgut, midgut cæca and the digestive tubules there is a uniform distribution of rod-like granular mitochondria throughout the cell. They are not clumped together (Fig. 8). In the case of the basal cells of midgut, midgut cæca and digestive tubules the mitochondria are in the form of small granules towards the inner half of the cell. These are very closely packed together (Fig. 9).



(5) *Golgi bodies*.—The Golgi elements in the young secretory cells (Fig. 10) are rod-like towards the middle region and towards the border next to the lumen they are curved and some of them in this region are in the form of rings. In older secretory cells with formed secretion, Golgi elements are absent from the basal region (Fig. 11). They are found towards the middle and distal regions. They are in the form of curved rods and rings.

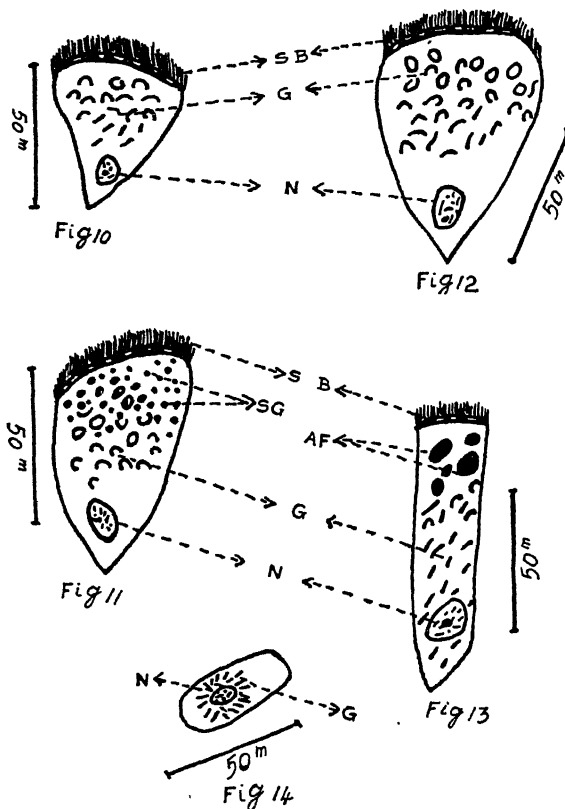
In the Mann Kopsch preparations stained with acid fuchsin the secretory granules stained faintly ; but darkly staining rims were present.

In secretory cells containing mature secretory granules the Golgi elements are very small and few in number. These observations suggest that the development of secretory granules in the zymogen region takes place in close association with and very often surrounded by Golgi elements.

In the case of secretory cells which have discharged their secretion into the lumen and which are to enter another phase of secretory activity, large number of complete rings of Golgi elements are found towards the region next to the lumen while towards the middle region, only a few rod-like Golgi elements are found (Fig. 12).

In the absorption cells, the Golgi elements which are in the form of rods are uniformly distributed. But they are somewhat crowded around the nucleus (Fig. 13).

The Golgi elements in the case of the basal cells are in the form of small rods and are grouped around the nucleus (Fig. 14).



*Discussion.*

Before the year 1924, a number of workers stated that the Golgi apparatus was the centre for the synthesis of the early secretion or pre-zymogen. They also observed that this early secretion stains with neutral red. Bowen (1926) observed that the act of secretion is clearly that by which the secretory granules are formed, the granules being to the cytologist, secretion. It was pointed out by him that mitochondria, nucleus and nucleolus play some part or other in the origin of the material of secretion from the cytoplasm and that this material is transformed into mature secretion by the activity of the Golgi elements. According to him, the primary elaboration of the secretion is due to the activity of the Golgi apparatus. Ludford and his co-workers (1925, 1926, 1929) agreeing in the main as to the synthetic activity of the Golgi apparatus extended the view still further to mean the production of intracellular enzymes. But Bowen (1926) throws out the 'most engaging hypothesis to extend the synthetic activity of the Golgi apparatus' as pure speculation. Beams and Goldsmith (1930) stated that when the secretory granules were first formed, they were associated with and surrounded by the Golgi elements. But they did not consider the Golgi elements as mainly responsible for the synthesis of the secretory material. Beams and King (1932) were also unable to see the part played by the mitochondria or the Golgi elements in the synthesis of the secretory granules. It was Michaelis (Duthie, 1933) who first pointed out that the prezymogen or early secretion arose near the mitochondria in the basal region of the cell. This migrated into the zymogen region. The work of Hirsch (1931) and Duthie (1933) confirmed the observations of Michaelis (Duthie, 1933). The early secretion came into association with Golgi elements as it moved from the prezymogen to the zymogen region. Thus the Golgi elements are not the primary centres of elaboration of the secretion. The secretion only comes into the region of the Golgi elements at a much advanced stage of its development.

Gresson (1934) also suggested that the prezymogen arises under the influence of the mitochondria in the basal region of the epithelial cells. It next moves into the region of the nucleus and finally to the region of the Golgi elements in whose association, the secretory granules become matured.

In *Paratelphusa* the material of secretion is separated from the ground cytoplasm in small vacuoles under direct influence and association of the mitochondria in the prezymogen region. These vacuoles take up neutral red. In cells in which the secretory vacuoles have not yet appeared the mitochondria are larger in number. With the appearance of the secretory

vacuoles the mitochondria decrease in number and the hitherto filamentous mitochondria in addition to the influence they exercise in the separation of the secretory vacuoles from the ground cytoplasm, might also, by direct transformation, contribute in part to the formation of the secretory material. The neutral red staining vacuoles then move away from the prezymogen region into the zymogen region. Here they are closely associated and surrounded by the Golgi elements. Even now the secretory material is stained by neutral red. In the later stages the vacuoles are converted into granules by the deposition of material within their interior under the direct influence of the Golgi elements. This is suggested by the interior of the vacuoles which are stained by neutral red in varying degrees. The mature secretory granules decrease in size and are smaller. These do not stain in neutral red. When sections prepared by Mann Kopsch method are bleached and then stained in acid fuchsin, the faintly stained secretory granules are surrounded by dark rims constituting the Golgi body. It is quite evident that the maturation of the prezymogen into zymogen is chiefly brought about under the direct influence and intimate association of Golgi elements.

#### *Summary and Conclusion.*

1. The epithelial cells of the midgut and its cæca are entirely absorptive in function while the epithelium of the digestive tubules is composed of both secretory and absorptive cells occurring side by side.

2. The basal cells of the midgut, midgut cæca and digestive tubules are neither secretory nor absorptive. Their function appears to be the replacement of the epithelial cells when they later get degenerated after prolonged activity and during moulting as pointed out by Yonge (1924) and Gresson (1934).

3. Mitochondria in young secretory cells are filamentous and numerous. They are clumped together towards the region next to the lumen. In old secretory cells packed with secretory granules they are less numerous and are rod-like. They are absent from the region of the striated border. In old secretory cells without secretory granules they are in the form of filaments around the nucleus while they are granular towards the striated border. In the absorption cells they are rod-like, granular and are found throughout the cell. In the case of the basal cells they are found towards the inner half of the cells in the form of a number of granules which are very closely packed.

4. The Golgi elements in the young secretory cells are rod-like about the middle of the cell while they are in the form of curved rods and rings towards the region next to the lumen. In old secretory cells with secretory

granules no Golgi elements are found towards the basal region. A number of rings and curved rods are found in close association with secretory granules towards the region next to the lumen. In old secretory cells without secretion a few rod-like Golgi elements are found towards the middle, while some curved rods are placed towards the striated border. In the absorption cells, rod-like Golgi elements occur throughout the cell. They are crowded around the nucleus while only a few are found towards the region next to the lumen. In the basal cells they are found around the nucleus in the form of rods.

5. In the secretory cells, the prezymogen arises from the cytoplasm of the basal region of the cell in the form of small vacuoles, under the influence of mitochondria. It is quite probable that some of the mitochondria by their direct transformation might contribute to the formation of the prezymogen. The prezymogen then migrates into the region next to the lumen where it gets closely associated with the Golgi elements and is elaborated into zymogen granules under the direct influence of the Golgi elements.

6. The absorption cells absorb the food material from the lumen. The absorbed material is enclosed within the vacuoles present in the absorption cells.

In conclusion, I have to express my indebtedness to Dr. A. S. Rau, B.A., D.Sc. (London), for his kindness in going through the manuscript and suggesting many valuable improvements. My thanks are also due to Mr. K. V. Reddy, B.A., B.Sc. (Edin.), and Dr. S. G. M. Ramanujam, M.A., Ph.D. (London), for their kind interest.

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EXPLANATION OF FIGURES.

- FIG. 1.—A portion of the transverse section of a digestive tubule.  
FIG. 2.—A young secretory cell showing the formation of secretory vacuoles.  
FIG. 3.—Secretory cell at a later stage of secretion.  
FIG. 4.—Secretory cell with mature secretory granules.  
FIG. 5.—A young secretory cell showing the filamentous mitochondria.  
FIG. 6.—Secretory cell at a later stage with rod-like mitochondria towards the middle and the base.  
FIG. 7.—Secretory cell with mature secretion and filamentous mitochondria towards the base round the nucleus.  
FIG. 8.—An absorption cell with rod-like and granular mitochondria.  
FIG. 9.—A basal cell with granular mitochondria towards one pole.  
FIG. 10.—A young secretory cell with Golgi elements.  
FIG. 11.—Secretory cell at a later stage with Golgi elements and developing secretory granules.  
FIG. 12.—An old secretory cell without secretion and with Golgi elements towards the striated border and middle region.  
FIG. 13.—An absorption cell with Golgi elements and absorbed material.  
FIG. 14.—The basal cell with Golgi elements grouped around the nucleus.

REFERENCE LETTERS.

<i>A.C.</i> ..	Absorption cells.	<i>M.</i> ..	Mitochondria.
<i>A.F.</i> ..	Absorbed food material.	<i>N.</i> ..	Nucleus.
<i>B.C.</i> ..	Basal cells.	<i>S.</i> ..	Secretion.
<i>B.M.</i> ..	Basement membrane.	<i>S.B.</i> ..	Striated border.
<i>D.S.</i> ..	Detached portion of the cell with secretion.	<i>S.C.</i> ..	Secretory cells.
<i>F.</i> ..	Food material in the lumen.	<i>S.G.</i> ..	Secretory granules.
<i>G.</i> ..	Golgi elements.	<i>S.V.</i> ..	Secretory vacuoles.

# OBSERVATIONS ON THE ABNORMALITIES IN THE COMMON INDIAN FROG, *RANA TIGRINA* DAUD.

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[Communicated by Dr. G. S. Thapar, M.Sc. (Punj.), Ph.D. (Lond.)]

## *Introduction.*

THE frog is used throughout the world for class dissections, and many cases of abnormalities in its anatomy have been recorded on various occasions. Thus Howes (1888) described a case of the persistence of the left azygos vein in a female frog, while Parker (1889) gave an account of the occasional persistence of the left posterior cardinal vein in the same species and indicated the homologies of the veins with those of Dipnoi. Subsequently, this condition was noted with variations in details by Woodland (1910), O'Donoghue (1911 and 1912) and Collinge (1915), some of whom also tried to explain the significance of such variations. Lloyd (1921) gave an account of the occasional persistence of the right posterior cardinal vein in the adult *Rana temporaria* and of the abnormal genital organs in a female specimen. Later (1928) the same author recorded certain abnormalities in the vascular system of *Rana temporaria*. All these cases, however, concern the common European frog.

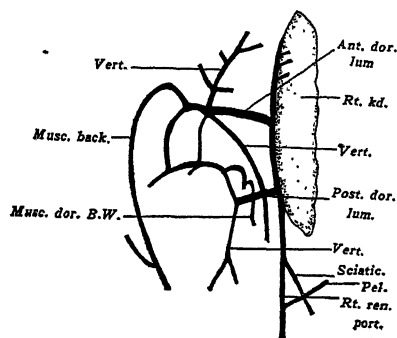
*Rana tigrina* is the common bull frog fairly abundant in these provinces, and is largely used for dissections in our classes. Some abnormalities in its anatomy have already been described. Crawshay (1906), while recording variations in the arterial system of Anura, made brief references to it. Nicholls (1915) described in two papers the variations found in its urostyle and anatomy of *Rana tigrina*. Bhattacharya and Das (1920) described a persistent oviduct in a male specimen, and Ahuja (1921) reported on the presence of a new vein and artery in *R. tigrina*. Bhaduri (1929) gave references to certain vascular abnormalities in the same species while recently, Mahendra (1936) has given a detailed account of a case of polymely in this species.

The present observations were made on a large number of specimens dissected in our junior classes. The abnormalities described here have not, as far as we are aware, been recorded so far. It is true that the variations to which the vascular system is liable lessens the importance, often ascribed to such abnormalities, but we hope that the present paper may not be without value as a contribution to the further study of the subject on a wider basis than is indicated here.

I.

*Presence of Two Dorso-Lumbar Veins.*

*Material and Observations.*—Out of the sixteen specimens dissected, one specimen possessed two dorso-lumbar veins instead of one (Text-Fig. 1). Similar multiplicity of these veins has been mentioned by Ecker and Haslam (1889) and Gaupp (1899) for *R. esculenta*. The specimen was an adult female



TEXT-FIG. 1.

The right renal portal vein of the abnormal specimen, showing the origin of the double dorso-lumbar veins.

*Ant. dor. lum.*, Anterior dorso-lumbar vein ; *Musc. dor. B. W.*, vein from the muscles of dorsal body wall ; *Musc. back.*, vein from the muscles of the back ; *Pel.*, vein from the pelvic region ; *Post. dor. lum.*, posterior dorsolumbar vein ; *Rt. kd.*, right kidney ; *Rt. ren. port.*, right renal portal vein ; *Vert.*, vein from the vertebral column.

of an average size. Further detailed investigation of the occurrence of this abnormality in a large number of specimens shows that whenever this abnormality occurs, it is found on one side only. There was not a single specimen in which we could find the presence of the two dorso-lumbars on either side.

The occurrence of the two dorso-lumbars has nothing to do with sex or size of the animal, as out of the three specimens showing this feature, two were males and one female. One of these three specimens was fairly large, while the other two were of an average size.

*Discussion.*—The first (*i.e.*, the anterior) dorso-lumbar vein joins the renal portal vein approximately between the anterior and the posterior ends of the kidney. It is formed by the confluence of several minute branches coming from the vertebral column, and a larger venule from the muscles and the body wall of this region. The second (*i.e.*, the posterior) is formed by the union of two venules, the anterior of which receives blood from the muscles of the back and from the vertebral column, and the posterior receives it only from the body wall. It is interesting to note that the two dorso-lumbar

veins anastomose with each other. The renal portal vein, just before meeting the kidney, receives a small venule from the pelvic region also.

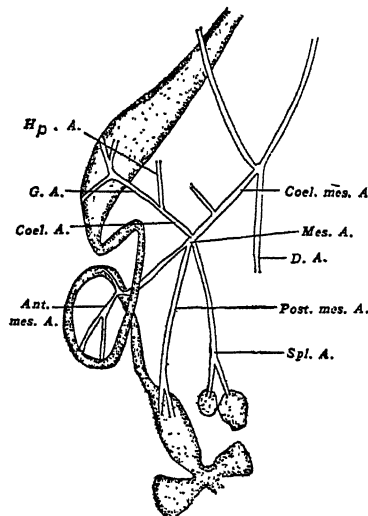
## II.

### *Presence of Abnormal Spleen.*

While trying an injection mass upon a specimen of the common Indian frog for dissection, we came across a rather peculiar abnormality in the structure of the spleen. This frog possessed a spleen divided into two separate lobes, one being smaller than the other. The bigger lobe has the size of a normal spleen, *i.e.*, 0.3 inches. A large number of preserved specimens were then examined and out of 50 specimens, only one showed this peculiar abnormality. In this case, however, one lobe was much smaller than the other. Out of the two specimens showing this abnormality, one was male and the other female. In the male specimen the two lobes were partially joined with each other, but in the female they were entirely separate.

*Blood Circulation.*—The arrangement of the blood vessels in the two lobes of the spleen was distinct and separate.

*Arterial.*—In a normal frog the spleen receives aerated blood through the splenic artery, which originates from the mesenteric artery. In the abnormal male (Text-Fig. 2), the splenic artery is the same, but here it bifurcates into two branches, each supplying one lobe of the spleen. In the female

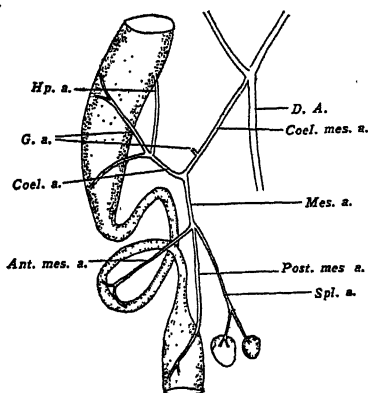


TEXT-FIG. 2.

Abnormal spleen and its arterial blood supply (male specimen).

*Ant. mes. A.*, anterior mesenteric artery; *Coel. A.*, coeliac artery; *G. A.*, gastric artery; *Hp. A.*, hepatic artery; *Mes. A.*, mesenteric artery; *Post. mes. A.*, posterior mesenteric artery; *Spl. A.*, splenic artery; *Coel. mes. A.*, coeliaco-mesenteric artery.

abnormal specimen (Text-Fig. 3), the branch of the splenic artery supplying the bigger lobe of the spleen again divides into two smaller arteries in the substance of the spleen.

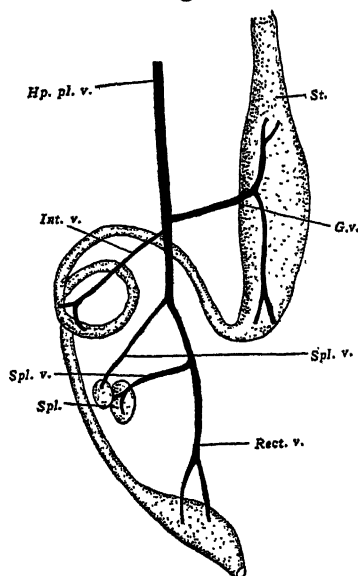


TEXT-FIG. 3.

Abnormal spleen and its arterial supply (female). Lettering as in Text-Fig. 2.

*Venous.*—In the normal frog a vein, running anteriorly from the rectum, passes just close to the spleen, and after collecting blood from it, joins the vessels from the intestines and the stomach to form the hepatic portal vein.

In the abnormal male specimen (Text-Fig. 4) however the arrangement is different. The capillaries in the larger lobe of the spleen combine to form



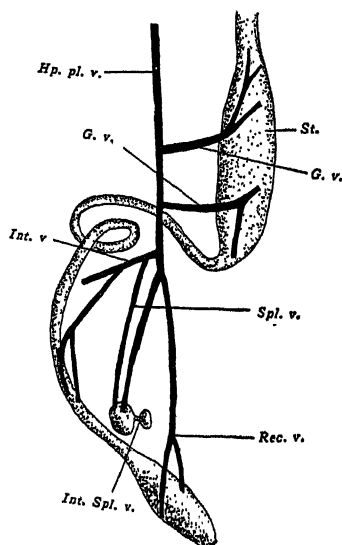
TEXT-FIG. 4.

Venus supply of the abnormal spleen (male specimen).

*Hp. pl. v.*, hepatic portal vein ; *Int. v.*, intestinal vein ; *Rect. v.*, rectal vein ; *Spl.*, spleen ; *Spl. v.*, splenic vein ; *St.*, stomach.

two small veins in the substance of the lobe. These two branches join as soon as they emerge from the lobe and the vein thus formed ultimately joins the rectal vein. The smaller lobe of the spleen, however, has a separate vein which runs forward and joins the rectal vein independently.

In the abnormal female specimen (Text-Fig. 5), the case is altogether different. The splenic lobes are inter-connected by means of a small blood vessel. Unlike the abnormal female, the smaller lobe does not send any



TEXT-FIG. 5.

Venous supply of the abnormal spleen (female specimen).

Lettering the same as in Text-Fig. 4.

*Int. spl. v.*, Inter-splenic vein.

direct branch to the rectal vein. On the other hand, two separate veins emerge from the bigger lobe. One of these, the inner one, runs up and joins the rectal vein. The other or the outer vessel runs more or less parallel to the inner branch and ultimately joins the intestinal vein. It is interesting to note that side by side with this abnormality, the female specimen showed the presence of the two dorso-lumbar in connection with the right renal portal vein.

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# OCCURRENCE OF STEM CANKER DISEASE OF SUGARCANE (*CYTOSPORA SACCHARI* BUTL.) IN THE PUNJAB.

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### 1. *Introductory.*

THE fungus *Cytospora sacchari* Butl. was first noticed in the Punjab at the Sugarcane Research Station, Risalewala, Lyallpur, on dried pieces of canes of several Coimbatore varieties lying about in the fields in March 1935. It was also observed, during the same year, as a saprophyte on cane Co. 244 in Rohtak and on Desi Ponda (thick chewing cane) in Lahore District. In 1936 the disease was reported from Mozaffargarh on Co. 223 as a doubtful parasite. In March 1936, the fungus was again observed when the seed canes of variety Co. 313 and some others, which had been buried in the ground since January to protect them from frost, were taken out and stripped for planting purposes. In the summer of 1936 the fungus appeared apparently as a distinct parasite on the standing crop at the Sugarcane Research Station, Risalewala, Lyallpur. Almost all the attacked canes wilted. The occurrence of the fungus has also been reported from Jhang, Gurdaspur and Ferozepore.

The fungus *Cytospora sacchari* Butl. was first recorded in India by Butler in 1906. From abroad it has been reported from Porto Rico by Petrak (1923); but apart from the description of the fungus on the host (Butler, 1906) no further work seems to have been done anywhere.

## 2. *Symptoms and Nature of Damage.*

The disease manifests itself in different forms. In its most serious form, the disease causes wilting of cane. The affected canes show drying up of the leaves from the tips downward as if suffering from drought. The cane stem gets shrivelled up and is poor in juice. Whole stools or only a few canes in a stool may be diseased. When the whole stool is affected, observations have shown that mother setts of such stools bear pycnidia of *Cytospora sacchari* Butl. Observations have further shown that when the stool is partially attacked the affected canes in addition show signs of damage by borers, rats or jackals. Under these conditions holes and wounds may facilitate the entry of the fungus. In such cases the attack of the fungus may be confined only to a few internodes, the portions of the cane above and below the affected internodes being healthy.

The pycnidia of the fungus are generally formed when the cane has completely dried up but in some cases pycnidia have been observed on partially green canes also. When the whole cane wilts, all internodes or only some of them may bear pycnidia. Pycnidia have been found even on buds at some badly diseased nodes and in the hollows of diseased cane stems. The fungus also attacks the leaf-sheaths and forms pycnidia on them. It appears, therefore, that the pycnidia of the fungus can appear on all parts of the above-ground portion of the cane. It may also be noted that the fungus becomes more virulent when the cane reaches maturity.

The fungus develops with great rapidity and severity when the canes are damaged by rats or jackals. Thus the canes which would otherwise give some yield are totally killed by the fungus. The disease also damages the canes which are buried for seed purposes. In the Punjab it is a general practice to bury canes reserved for seed purposes in the ground towards the end of December before the frost sets in and to keep them buried till sowing time, i.e., the month of March. The adoption of this measure is necessary with a view to protect canes from frost. If the fungus is present in the field, no matter in whatever form, whether as a parasite or as a saprophyte, the canes from such a crop when buried for seed purposes are attacked by this fungus and pycnidia develop in large numbers. The affected seed canes are altogether killed.

The structure of pycnidia and mycelium of the fungus on the host has already been described by Butler (1906) and need not be repeated here.

## 3. *Parasitism of Cytospora sacchari Butl.*

The fungus *Cytospora sacchari* was isolated from diseased canes of varieties Co. 312, Co. 313, Co. 323, Co. 371 and Co. 395, collected from the

Sugarcane Research Station, Risalewala, Lyallpur, and from canes of Co. 223 and Co. 285 collected from Mozaffargarh and Jhang respectively. All the isolations made from different varieties of cane collected from different localities were identical. The fungus was purified by monospore culturing.

Healthy canes of different varieties were inoculated with the culture of the fungus during 1936 and 1937. Inoculations were done by making holes in the cane stem by means of the cork-borer. Culture of the fungus was placed inside the hole and the hole plugged. In all cases controls were kept. These consisted of canes the stems of which were bored but were replugged without inserting the culture of the fungus. The details of inoculation experiments are presented in Table I.

TABLE I.

*Results of Inoculation Experiments on Standing Canes with  
Cytospora sacchari Butl.*

Date of inoculation	Variety of cane	No. of canes inoculated	No. of canes affected			Percentage of infection	No. of control canes	No. of canes affected	Percentage of infection
			Bearing pycnidia	Not bearing pycnidia	Total				
1. 21-12-1936 ..	Co. 323	20	2	18	20	100	20	0	0
2. 15- 7-1937 ..	Co. 312	16	6	10	16	100	10	0	0
3. 4- 9-1937 ..	Co. 371	20	16	4	20	100	10	0	0
	Co. 318	10	3	7	10	100	5	0	0
	Co. 313	11	4	7	11	100	5	0	0
	Co. 312	11	6	5	11	100	5	0	0
	Co. 373	20	2	18	20	100	5	0	0
	Co. 323	40	12	28	40	100	40	0	0
4. 19-10-1937 ..	(i) Co. 371	10	10	0	10	100	5	0	0
	(ii) Co. 371	10	2	8	10	100	5	0	0
	(iii) Co. 371	10	5	5	10	100	5	0	0

The results of these experiments prove that *Cytospora sacchari* Butl. is parasitic on sugarcane.

It may be mentioned that in the inoculation experiments no inoculated cane wilted. The infection spread above and below the point of inoculation to the extent of two to three internodes only upto 31st January 1937 in the

case of experiment No. 1 and 30th November 1937 in the case of experiments Nos. 2, 3 and 4.

The symptoms of the disease appeared 10 to 15 days after the date of inoculation and the earliest appearance of pycnidia was recorded after 17 days after the date of inoculation.

#### 4. Summary.

1. The occurrence of *Cytospora sacchari* Butl. is recorded in the Punjab.

2. Symptoms of the disease and nature of damage caused by *Cytospora sacchari* Butl. have been described.

3. The parasitism of the fungus has been proved on sugarcane by inoculation experiments.

The investigation reported was carried out as a part of the scheme of Sugarcane Research financed by the Imperial Council of Agricultural Research.

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# APOGAMY IN *ADIANTUM LUNULATUM* BURM, PART I (MORPHOLOGICAL).

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Received May 30, 1938.

[Communicated by Dr. H. Chaudhuri, D.Sc. (Lond.), Ph.D., D.I.C.]

THE genus *Adiantum* belongs to the Gymnogrammoid section of Leptosporangiate Ferns. Eight species of the genus out of 184 species known so far occur in India. *Adiantum lunulatum* is cosmopolitan being met with in the tropics of the whole world. In India it occurs very commonly in the South in plains and lower slopes of the hills and in the North along the foot of the Himalayas from East to West at an altitude of 1000–3000 feet.

The gametophytes were raised in cultures in two lots, (a) from spores brought from Sikkim in E. Himalayas in October 1931 and sown the same year in the middle of November and (b) from Kulu (Kataula-Mandi Road) in N. W. Himalayas in 2nd week of October 1933 and sown next year in November. In the latter case the spores lost their viability to the extent of 50%.

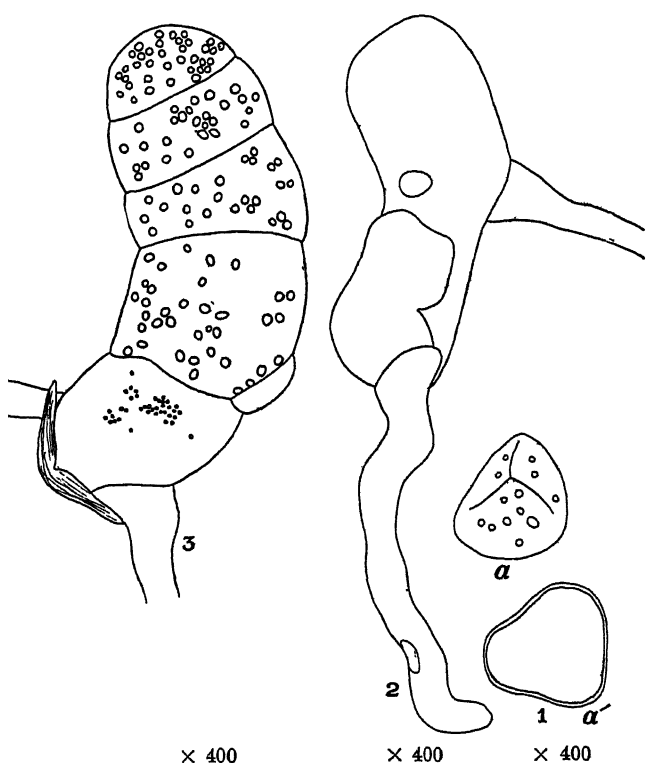
The cultures were raised on (a) sterilized soil in pots watered from below (by keeping the pot in a trough of water) and covered above with a glass plate to avoid contamination of foreign spores and kept in a glass house and (b) on standard Knop's solution in sterilized petri dishes. The germination of spores is earlier in Knop's solution taking about 12 days than on soil which takes about 2–3 months. The most vigorous growth occurs in the month of March.

The earlier stages of germination were drawn from fresh material. Permanent preparations of fully developed prothalli were made in Canada balsam after staining with safranin and gentian violet.

## *Description.*

The spores are  $47\mu$ – $51\mu$  in diameter, dark brown in colour, tetrahedral with tri-radiate mark and possess two coats—a thin intine and a comparatively thick exine (Text-Fig. 1 a, a'). The exine is smooth as in other species of the genus like *A. caudatum* L., *A. capillus-veneris* L., etc. The spores are devoid of chlorophyll and contain numerous oil globules of different sizes.

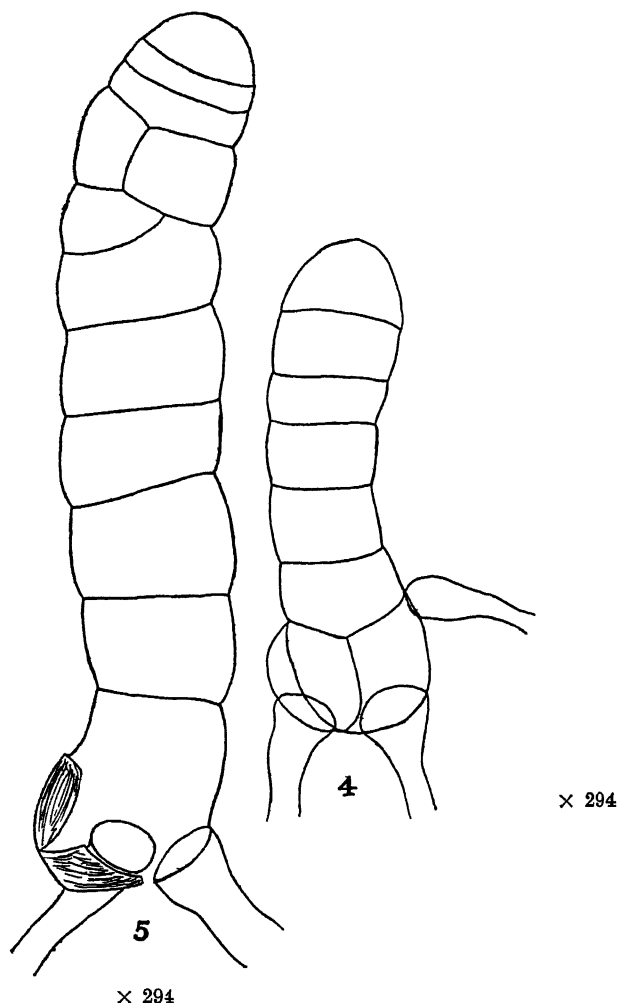
The primary stages of germination are as usual for ferns. The spore ruptures at the tri-radiate mark giving out a papilla in which chlorophyll develops. A rhizoid or frequently two are given off early from its sides (Text-Fig. 2). Transverse walls are then laid in the papilla forming a filamentous protonema 2-8 or 9 cells long dependent on the moisture and light conditions of its growth (Text-Figs. 3, 4, 5). In favourable light the protonema begins expansion even in the second cell but in feeble light and enough moisture it may become 9 to 10 cells long and may even show branching. Sometimes the basal cell of a protonema becomes much swollen. In Text-Fig. 4 such a cell has cut off a subsidiary cell on one side bearing a rhizoid.



The basal cell (and frequently but not invariably the next upper cell also) shows only a few chloroplasts of very small size in contrast to the large fully developed chloroplasts packing the upper cells of the protonema (Text-Fig. 3).

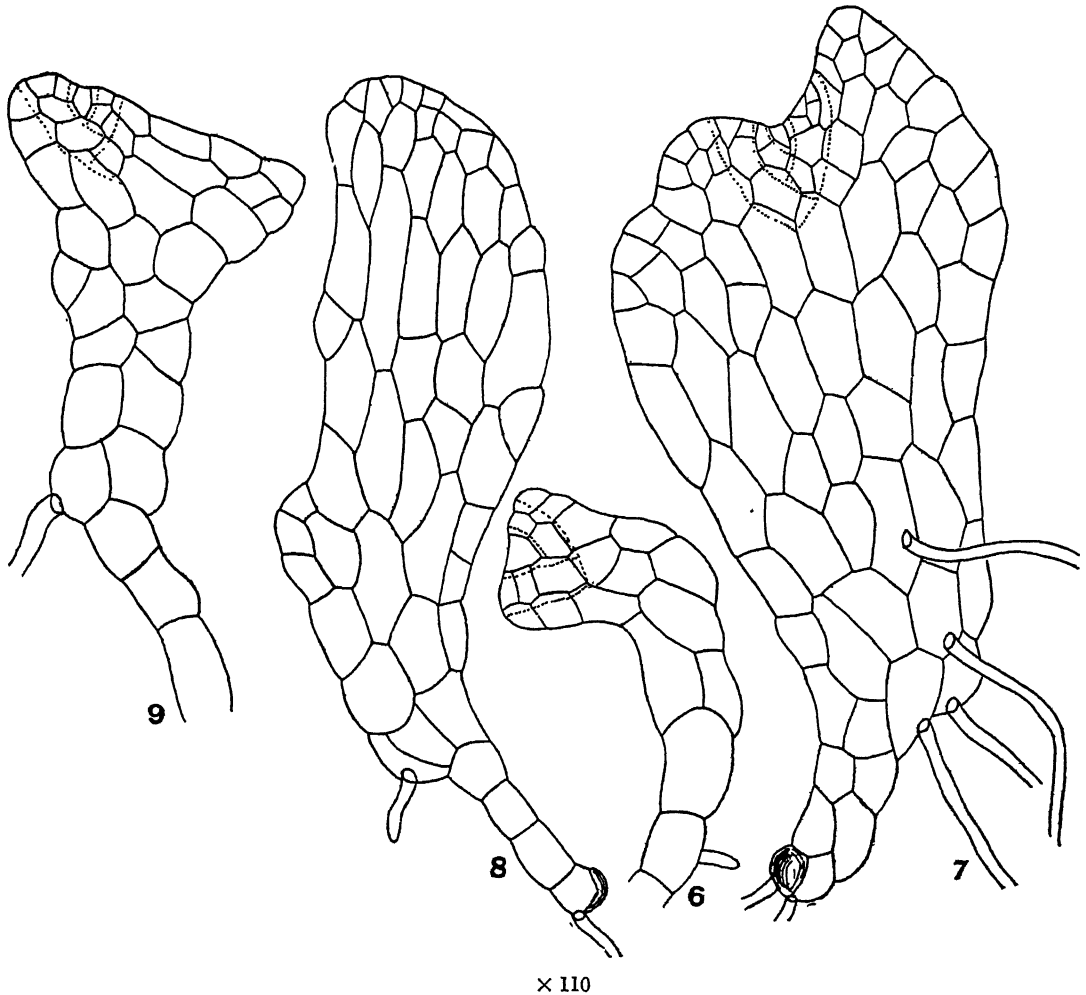
The expansion of the gametophyte takes place by the formation of a two-sided apical cell formed from the uppermost cell of the protonema in the manner usual for ferns. Frequently any of the lower cell of the filament

may become two celled by the formation of a longitudinal wall before any cleavage occurs in the apical cell (Text-Fig. 5).



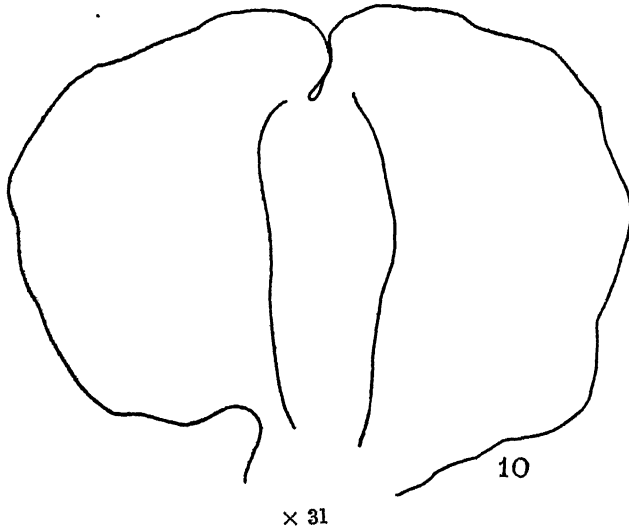
The two-sided apical cell cuts off segments alternately on either side (Text-Fig. 6), resulting in a cordate type of prothallus with the two lobes of the same age and equally developed (Text-Fig. 7). Later, this cell is cut into an apical meristem of brick-shaped cells by anticlinal walls as usual in ferns. The further growth of the gametophyte occurs through the activity of this meristem.

This behaviour described above does not invariably hold true for the species. Frequently the protonema flattens by the formation of periclinal



and anticlinal walls without any regular sequence into a spatula-shaped prothallus a single layer of cell in thickness (Text-Figs. 8, 9). A two-sided cell makes its appearance obliquely in the front which cuts off a few segments on either side (Text-Fig. 9). This is early cut into brick-shaped cells by anticlinal walls and it is by the activity of this true meristem that a smaller new lobe is formed. At a certain stage in the development, the prothallus appears more or less lop-sided with a bigger older lobe and a smaller younger lobe. Later the two lobes become equal in size due to the greater meristematic activity in the cells of the younger lobe and the prothallus becomes normally cordate (Text-Fig. 10). This type of growth, however, is not exactly what is recorded by Goebel for *Pteris longifolia* L., and observed by the writer in

*Pteris biaurita* L. and *Ceropteris calomelanos* L. (unpublished) in which the meristem develops in strictly lateral position in the spatula-shaped prothallus but may be regarded as intermediate between the normal type and *Pteris longifolia* type.

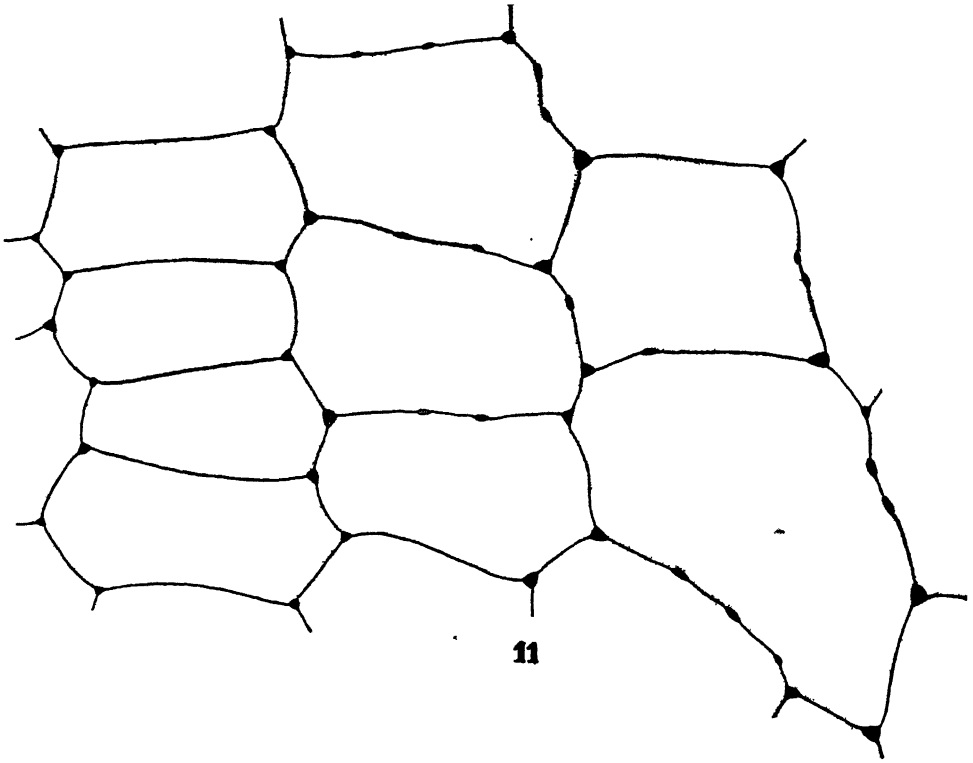


A cushion develops rather early in the history of the gametophyte in the axial region (Text-Fig. 10). *Adiantum caudatum* L. prothalli raised in culture by the writer along with those of *A. lunulatum* showed a remarkable contrast in that they did not form a cushion even after one year's growth. In the young prothalli in *A. lunulatum* the cushion is uniformly composed of isodiametric parenchyma. During later growth the cells of the anterior region of the cushion behind the notch become very much elongated, about 6-8 times their breadth, in the direction of the long axis of the gametophyte. The central cells of this region become comparatively narrower and more elongated than those on the sides which progressively towards the outside become shorter and broader till they merge into the isodiametric cells of the wings. During this elongation a change is brought about in the position of the apical meristem. It may become pushed out in the depression as a middle lobe (Plate VI, Fig. 6), which itself may become heart-shaped by the activity of the cells on either sides of the central region. But in most cases the meristem is deflected to one side thus assuming a lateral position (Plate IV, Fig. 2). It may become further shifted away from the notch by the greater division of the cells on the side of the notch in an anticlinal plane. In all these cases, however, a new cushion is formed behind the apical meristem which may or may not become continuous with the old cushion.

An important characteristic of the species is the presence of fully developed tracheids in the gametophyte as a normal feature. This feature was first observed in 1932 in the cultures raised from Sikkim spores (2). In order to ascertain that this character is of specific importance and not developed from the spores of some stray abnormal individual of the species of which the spores happened to be collected in Sikkim, a new culture was raised from spores collected from a widely different locality, *i.e.*, Kulu in N. W. Himalayas. These gametophytes also showed tracheids in their tissue. *Thus this feature may be regarded as a character of specific importance.* The tracheids are fully developed, long and with only spiral thickenings on their walls (Plate V, Figs. 3, 4, 5). They are formed in the anterior region of the cushion (the posterior region is almost invariably devoid of these) by the transformation of the elongated cells of the cushion mentioned above. The tracheids may be formed in a continuous row or may be disconnected. The posterior elements are usually broader, thicker and with compact spirals but those formed in front possess thinner walls, are narrower with lax spirals (Plate V, Fig. 3). The vascular elements formed just behind the apical meristem are more of the nature of tracheidal cells. Plate IV, Fig. 2, shows a prothallus in which the meristem has become deflected to one side. Dark streaks are observed in this at the regions marked A, B, C. Magnified photographs of these regions, Plate V, Figs. 3, 4, 5, respectively show that the tracheids are developed in this gametophyte at three different regions and that these are disconnected. The maximum development of the vascular elements is in the central cushion region A.

Gametophytes grown submerged in water from the early stages become elongated and strap-shaped and do not form any cushion. In these gametophytes tracheids are never formed. Normally developed gametophytes possessing tracheids when submerged in water, during further growth, form strap-shaped thalli and do not develop any further tracheids. It is, therefore, obvious that the gametophyte of *Adiantum lunulatum* possesses the potentiality of forming vascular tissue under normal circumstances under subaerial conditions and not under water.

The cells of the wing are polygonal. Those in the anterior region of the gametophyte show characteristic collenchymatous thickenings at the angles and also along the walls where, however, they are relatively poorly developed (Text-Fig. 11). No such thickenings are met with in the posterior region of the wing. Similar thickenings but more strongly developed have been observed in some other Gymnogrammoid ferns, in *Adiantum caudatum* L. and *Cheilanthes farinosa* Kaulf by the writer and *Adiantum cuneatum* L. and F., *Cheilanthes* and *Notholaena* by Horvat (quoted by Bower in *Filicales* III, p. 96).



× 600

*Sex Organs.*

Antheridia appear on very young prothalli. The precocity of their development depends on the environmental conditions. In crowded cultures in rather feeble light with enough moisture they are formed on quite young prothalli even in the filamentous stage and to such an extent that the prothalli get exhausted and afterwards die. In fairly developed prothalli, the antheridia may develop near the margins but usually in the cushion region on the upper or lower surface but usually the latter.

The structure of the antheridium is as usual for Leptosporangiate ferns. There is a basal funnel-shaped cell, a wall cell and an opercular cell which is thrown off bodily during antheridium dehiscence. The number of spermatids per antheridium varies from 22–38. In the low output of spermatids per antheridium the species resembles other ferns high up in the scale of evolution. The spermatozoids are quite normal in appearance and apparently quite capable of performing fertilization.

The appearance of archegonia is variable in the cultures from two different localities. The culture from Sikkim spores did not show any trace of

archegonia on any prothallus. In the other culture from Kulu spores a number of archegonia appeared in the cushion region of all the prothalli on the under surface. These possess the usual posteriorly curved neck and are otherwise quite normal in structure and appearance. This difference of behaviour in the formation of archegonia in the two cases is interesting. It may be added that both the cultures were raised in the same glass house, so that if it is at all due to external causes, which seems highly unlikely, it must have been because of the seasonal difference in the two years. It may be added that other fern prothalli cultivated alongside with the Sikkim culture of *Adiantum lunulatum* in 1931 developed normal archegonia in the usual manner.

#### *Embryo Formation.*

The embryo formation in the species takes place invariably in the form of apogamous bud. In Sikkim culture the complete elimination of archegonia rules out the possibility of any sexually developed embryo. Even in Kulu culture the archegonia play no rôle in the formation of embryo but shrivel off.

One or two or sometimes three buds make their appearance (Plate VI, Figs. 6 and 7), but usually one or in some cases two embryos come to maturity on a prothallus. The buds develop in the cushion region behind the notch usually from the under but also from the upper surface. Sometimes the first leaf appeared from the under surface while the other parts developed from the upper.

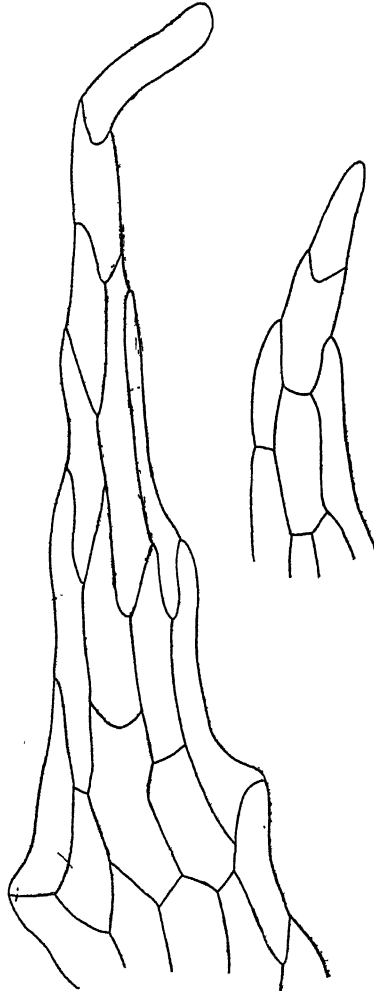
A few cells take part in the formation of the bud. If the initiation takes place just behind the apical meristem, the small cells recently formed are involved and divide actively, if it occurs in the slightly posterior region, the mature cells of the region concerned become highly meristematic. The result is the formation of a small celled tissue hemispherical in form. Tracheids are soon formed in this tissue but they are usually independent of the vascular elements of the prothallus.

An important feature to note is that *each cell of the embryonal tissue contains a single nucleus and at no stage any irregular doubling of the nuclei is observed in the cells of the embryonal tissue.*

The first leaf starts in the form of an apical cell cut off from the embryonic meristem and grows out anteriorly (Plate VI, Fig. 8). Soon after or sometimes before the formation of leaf-initial multicellular hairs appear on the embryonic tissue. These emerge at first as simple protuberances which become multicellular by the formation of transverse septa. Later on by the formation of longitudinal and transverse walls in their basal region, these hairs are transformed into flattened scales (Text-Fig. 12). A root is given

out in the posterior part of the prothallus and a stem apex closely surrounded by the scales completes the formation of an embryo (Plate VI, Fig. 9).

An interesting feature is that the first leaf may be simple (Plate VI, Fig. 9) or compound (Plate VI, Fig. 10). It may be mentioned that the first leaf formed from a fertilized ovum in the normal life-cycle of a fern is invariably simple.

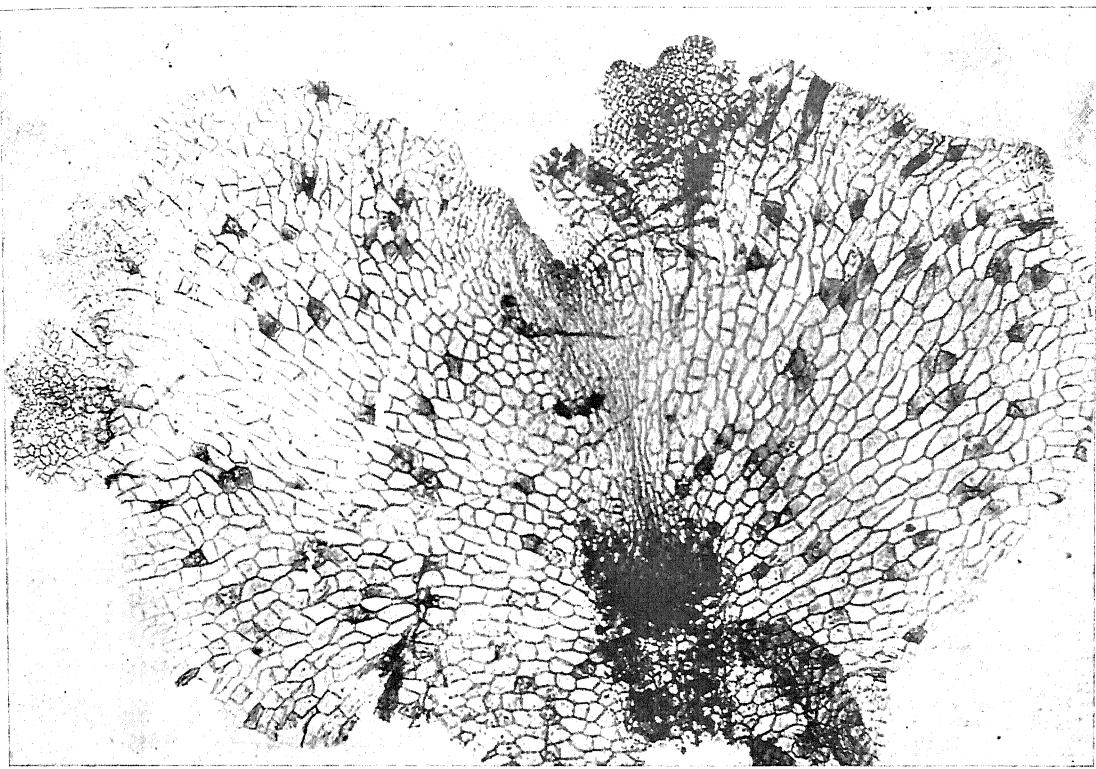


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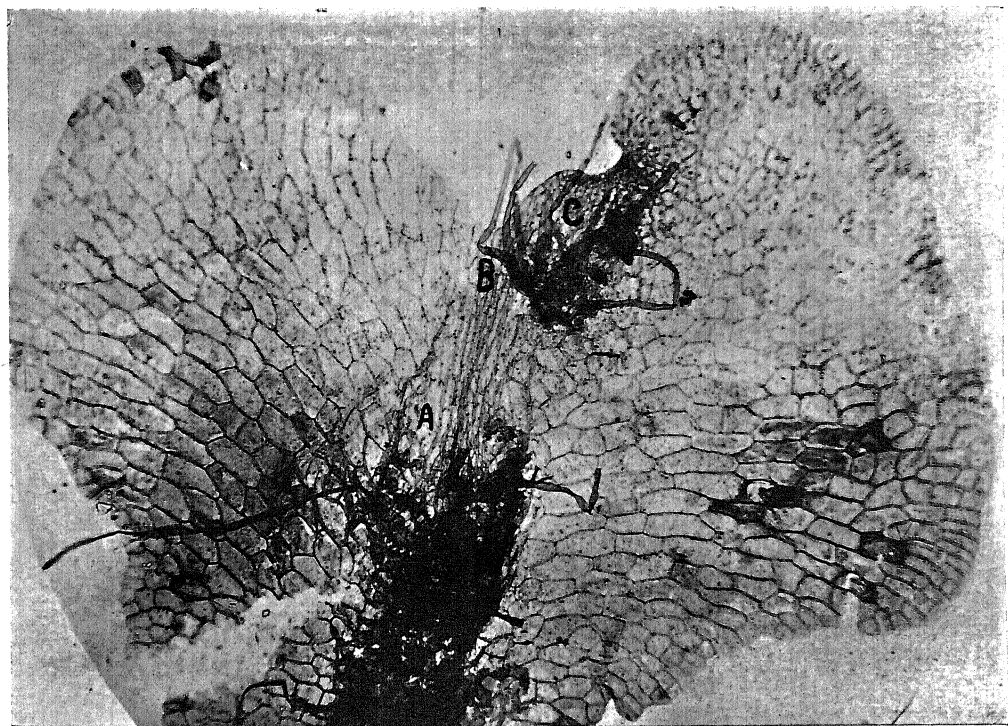
× 267

*Proliferation.*

The prothallus of *Adiantum lunulatum* that forms an embryo may further grow for some time, add some more tissue in front and even form a few



1



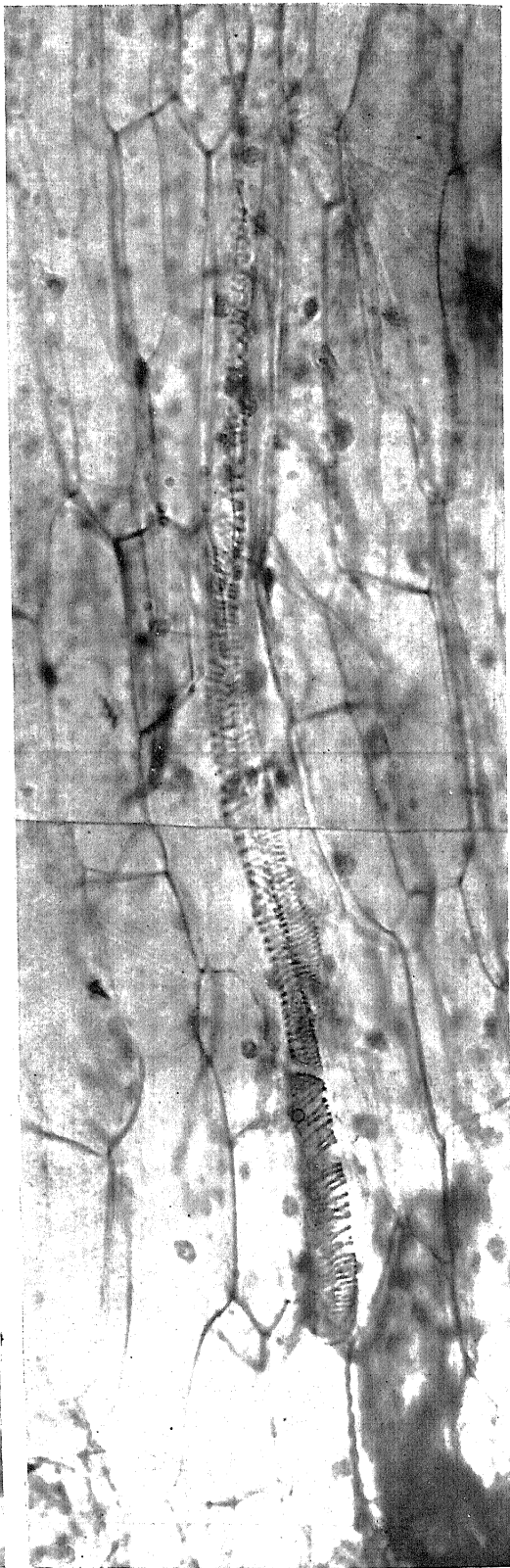
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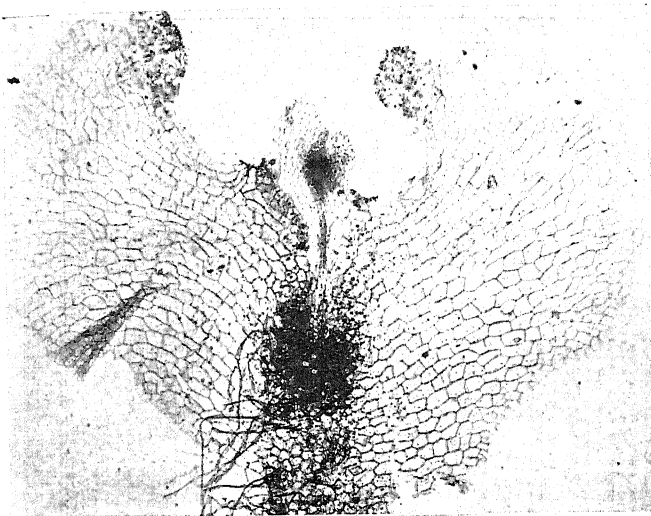
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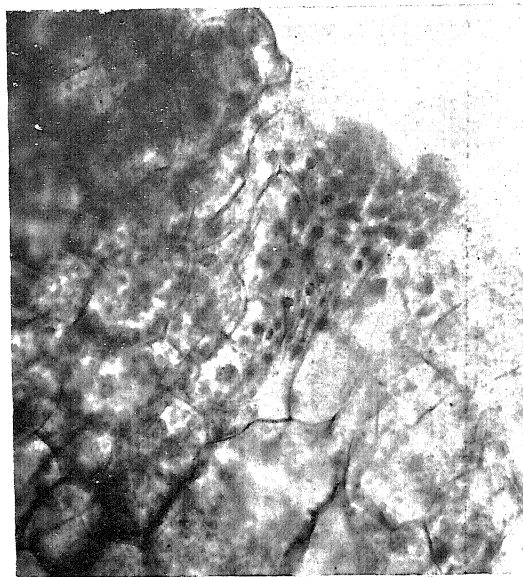
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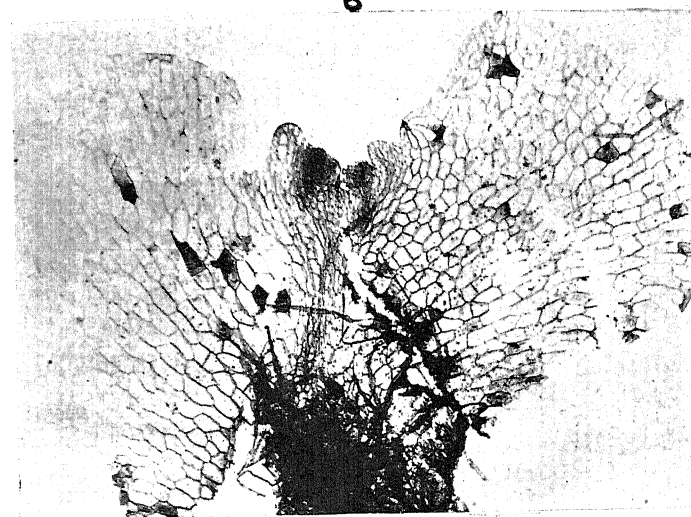
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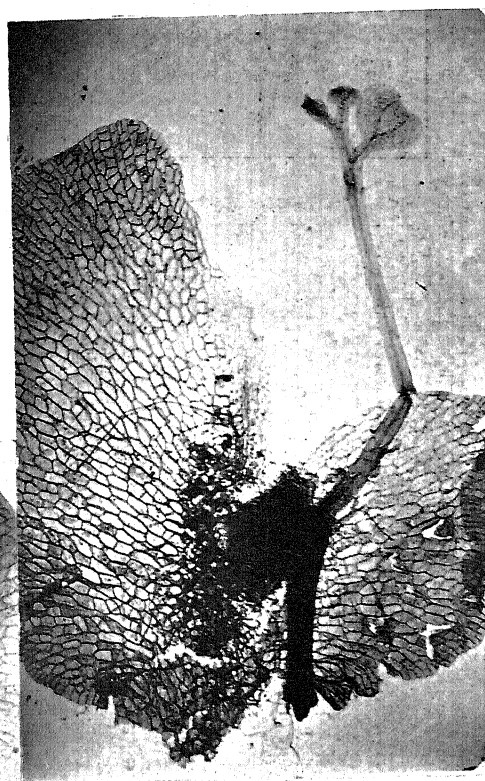
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10



9



tracheids inside but ultimately dies of exhaustion after the nourishment of the embryo. Even those that do not form any embryo live only for one season in the sense that they do not store up food material in their cushion as occurs in *Anisogonium esculentum* Presl. studied by the writer (unpublished) which thus perennates the unfavourable conditions and grows in the next season. Such prothalli, however, frequently proliferate at their margins giving rise to a number of daughter prothalli. Four such fresh growths are observed in an old prothallus of the species in which a number of cells in the wing appearing dark are already dead (Plate IV, Fig. 1).

A group of cells or even a single cell may serve to form a prothallus. In similar old but very massive prothalli of *Anisogonium esculentum* Presl. and *Nephrodium molle* Desv. the writer has observed a number of daughter prothalli formed on the dorsal surface of very thick cushion and also on the margin of the wings.

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#### EXPLANATION OF PLATES.

##### PLATE IV.

- FIG. 1.—An old prothallus proliferating at 4 different places along the margin. Dark cells are dead.
- FIG. 2.—A mature prothallus where the notch meristem has become side deflected through the elongation of the cells of the anterior region of the cushion. Regions marked A, B, C showing dark streaks contain tracheids.

##### PLATE V.

Highly magnified views of regions A, B, C of the prothallus shown in Fig. 2, Plate IV.

- FIG. 3.—Region A (anterior cushion region), Tracheids in a single row, those in the posterior part better developed.
- FIG. 4.—Region B. Under the two rhizoids an elongated tracheid is observed.
- FIG. 5.—Region C. A few tracheidal elements behind the notch.

##### PLATE VI.

- FIG. 6.—An apogamous bud developed just behind the apical meristem. The entire region has been pushed out by the elongation of the cells of the anterior cushion region. Dark streak contains the tracheids.
- FIG. 7.—Two apogamous buds on a prothallus.
- FIG. 8.—An apogamous bud region highly magnified. Brisk meristamatic activity of the cells, leaf initial in the form of a papilla.
- FIG. 9.—An apogamous embryo with cotyledon and root surrounded by scales.
- FIG. 10.—An apogamous embryo with compound first leaf or cotyledon.

# APOGAMY IN *PTERIS BIAURITA* LINN.

BY P. N. MEHRA.

(Kashyap Research Laboratory, Panjab University, Lahore.)

Received June 25, 1938.

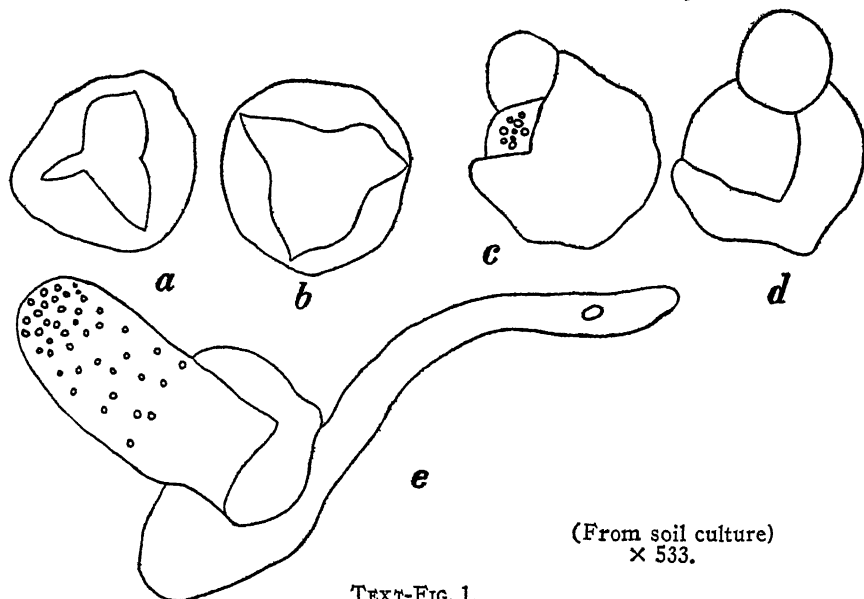
[Communicated by Dr. H. Chaudhuri, D.Sc. (Lond.), Ph.D., D.I.C.]

*Pteris biaurita* belongs to the Pteroid section of the Leptosporangiate ferns. The species is common in India in the Eastern Himalayas from Garhwal to Bhotan and Khasia ascending upto 6000 ft. The spores were collected in Sikkim where they ripen in the month of October.

The spores are tetrahedral, dark brown and possess a tri-radiate mark. As in other members of Pterideæ, the spore wall consists of a thin intine and a thick outer exine. There is a band of thickening developed on the periphery of the spore running along its three corners.

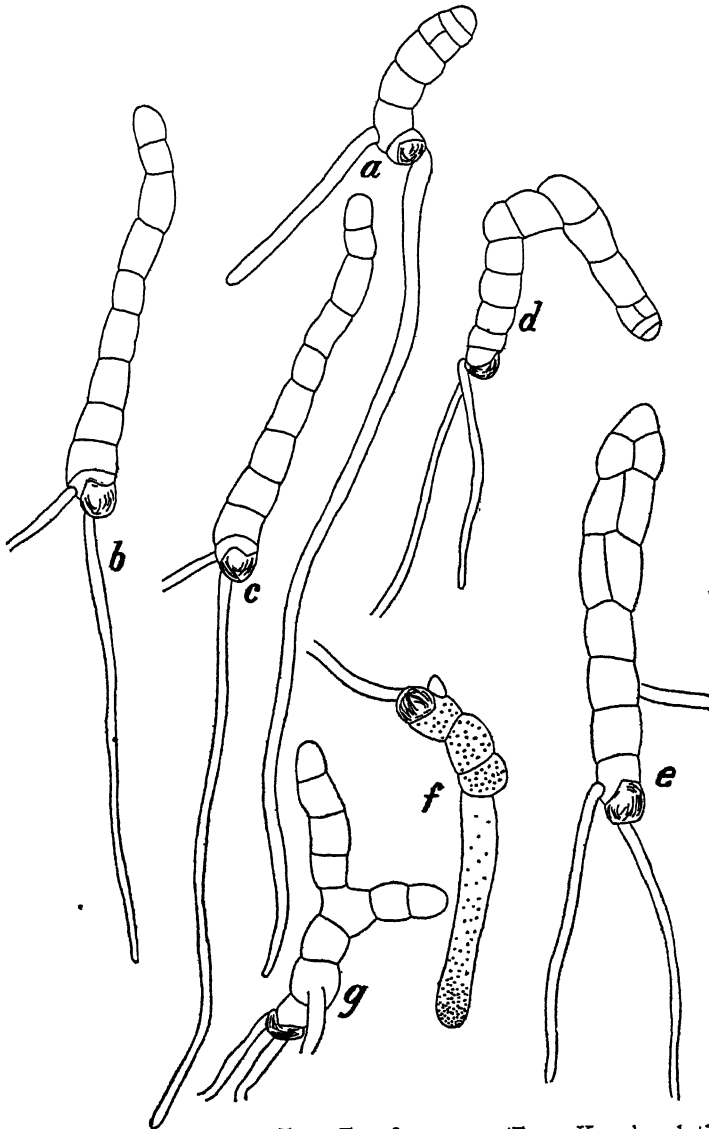
The spores were germinated on sterilized soil in pots kept in troughs of water and covered over with glass plates to avoid foreign contamination and in sterilized Petri-dishes on Knop's solution. The initial stages of germination in the latter medium are secured earlier in about 12 days than on soil which takes about 23 days. Drawings of the early germination stages were made from the living material from cultures from both the sources and these are specified. The later stages, after the protonema were, drawn exclusively from the soil culture.

The spores do not lose their viability even after an year.



TEXT-FIG. 1.

The spore swells in size and the exine ruptures at the tri-radiate mark (Text-Fig. 1 *a, b*) showing a number of oil globules and other food grains enclosed within the intine. The protuberance or the protonema initial gradually develops chlorophyll granules. At the same time primary rhizoid is cut off by a longitudinal or oblique wall (Text-Fig. 1 *c, d*). In the protuberance the food material and the chloroplasts collect in the upper region so that the lower part within the spore coat becomes empty and hyaline (Text-Fig. 1 *e*). Successive transverse walls are laid forming a protonema of variable length which in Knop's solution may be upto 13 cells long (Text-Fig. 2 *a, b, c*). In the meantime another rhizoid may be formed from



TEXT-FIG. 2.

(From Knop's solution)  $\times 95$ .

the lowermost cell or the one just above it. The growth of the rhizoids is much pronounced and smooth in Knop's solution than on soil. In the former medium the rhizoids develop a few small chloroplasts probably because of their being exposed to diffused light in the aquatic medium.

Frequently the protonema may branch (Text-Fig. 2 g). Sometimes in Knop's solution it forms a rather narrow elongated cell in front in which the chlorophyll grains become aggregated in the apical region (Text-Fig. 2 f).

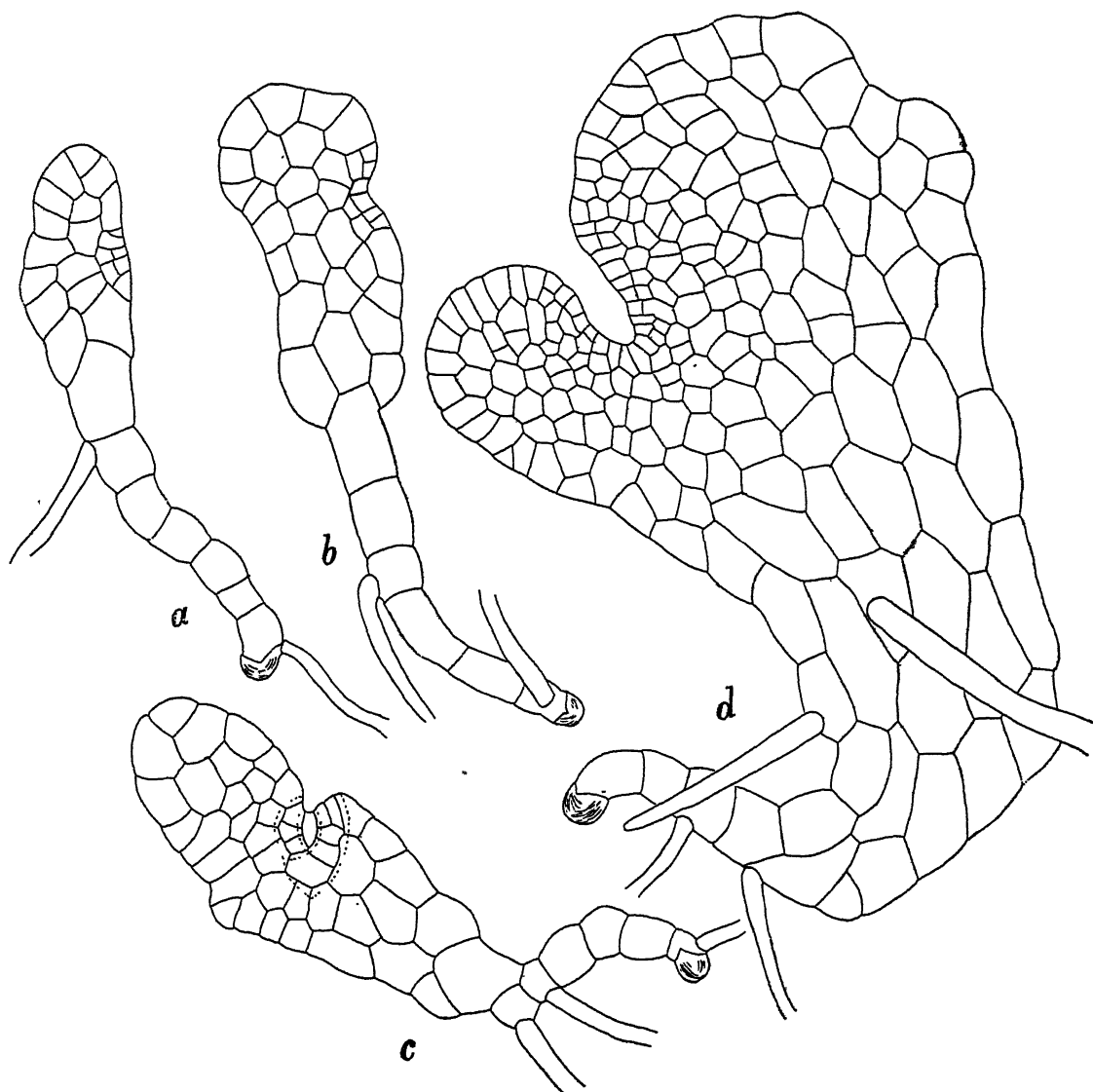
*Flattening of the gametophyte :—*

The prothallus in *Pteris bicaurita* does not expand by a two-sided apical cell.

The filament grows out in its anterior region into a spatula-shaped structure, one layer of cells thick, by the formation of vertical walls in the marginal and surface cells. One of the cells on the lateral margin divides into a number of smaller cells by periclinal, anticlinal and oblique walls (Text-Fig. 3 a). During this procedure a two-sided cell is differentiated which, as will be seen later, marks the position of the notch (Text-Fig. 3 b). It cuts off a few segments alternately on the right and left. These segments divide by both periclinal and anticlinal walls and grow outwards both above and below the two-sided lateral cell where a notch is formed (Text-Fig. 3 c). Later the segments below this two-sided cell begin to grow vigorously with the result that a second small lobe is differentiated. The prothallus at this stage is lop-sided with two lobes of unequal size (Text-Fig. 3 d). At the same time two-sided cell becomes dissected into more or less rectangular cells, by the formation of anticlinal walls, forming a short-celled meristem at the notch. The growth of the secondary smaller lobe is now more rapid compared to the big primary lobe. Ultimately the prothallus assumes a cordate appearance (Pt. VIII, Figs. 5, 6) with two lobes equal in size. It must be noted, however, that the two lobes are not of the same age.

This type of prothallial development resembles with that described by Goebel (1930) for *Pteris longifolia* and by Schumann (1915) for *Acrostichum aureum* both belonging to Pteridæ. In *Pteris flabellata* probably a similar thing happens as indicated by the figures of the apogamous prothalli drawn by Steil (1933). Similar development of the prothallus has been observed by the writer in a Gymnogrammoid fern *Ceropteris calomelanos* (unpublished).

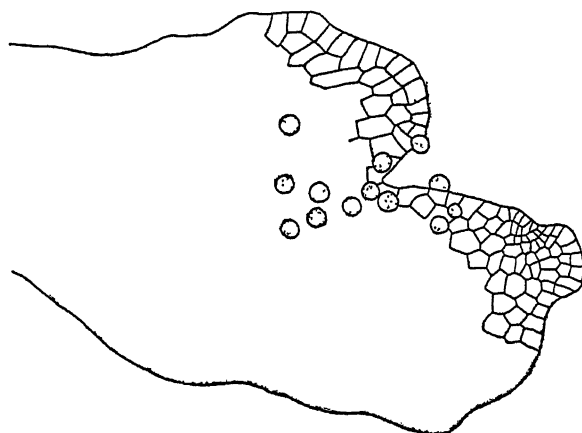
Frequently the lateral meristem arises very late. In such cases the prothallus forms a flat plate of cells of considerable size, one layer thick, and may commonly become lobed simply by the greater growth of cells on one



x 95

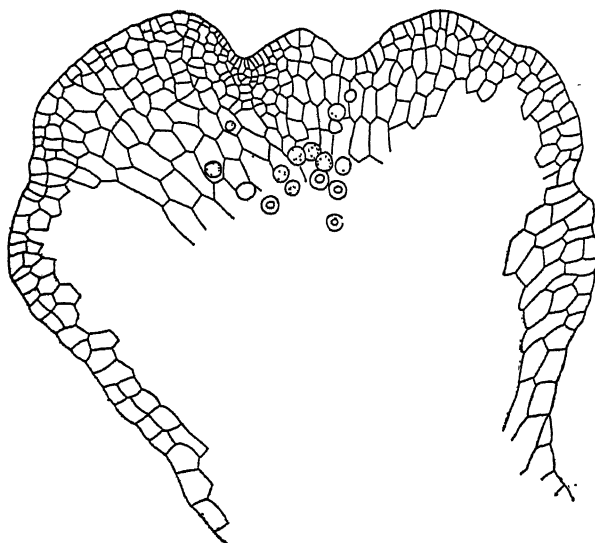
TEXT-FIG. 3.

side than on the other (Text-Fig. 4). Also the two-sided cell and later the meristem in these cases may arise obliquely anteriorly so that the second lobe is not formed entirely *de novo*, but is also to a certain extent a part of the original plate like thallus (Pt. VII, Fig. 1, Text-Fig. 5).



TEXT-FIG. 4.

× 46



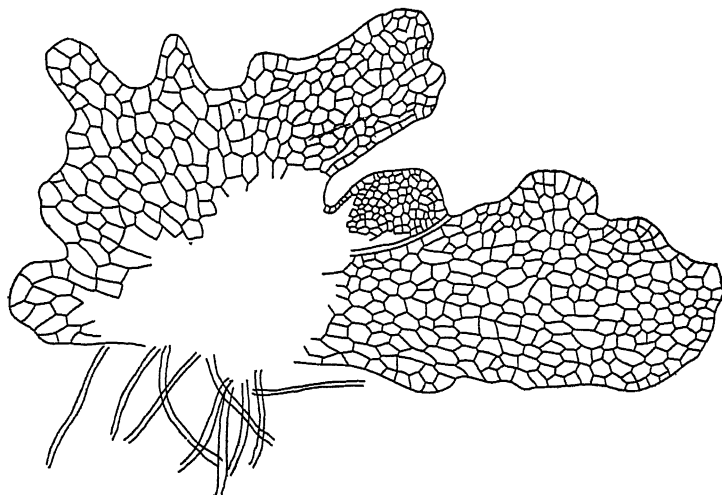
TEXT-FIG. 5.

× 46

These irregularities, however, are not very common. They serve to point out the plastic nature of fern prothalli.

In a few cases, a highly irregularly lobed prothallus was found to develop. One of the peculiar features in these is the formation of an elongated narrow cell separating two of the lobes, while on both of its sides smaller cells are present (Text-Fig. 6).

The mature cordate prothalli in the present culture on soil are, as a rule, a single layer of cells thick throughout, the typical well-developed cushion



TEXT-FIG. 6.            × 28

between the wings being absent. In *Pteris longifolia* a cushion is present. The fully developed cells of the wings do not possess any collenchymatous thickenings on their corners or along the walls such as have been observed by the writer in *Adiantum lunulatum* (1938) and *Cheilanthes farinosa* (unpublished) and described by Horvat in *Adiantum cuneatum* and Bauke in *Anemia phyllitidis* (quoted by Bower, 1928).

Antheridia develop on young prothalli in quite large numbers, particularly on the under surface but also along the margins and on the upper surface. Sometimes every cell of the young prothallus bears an antheridium. These prothalli get exhausted and die. Those which either produce no antheridia or only a few ones grow big, develop the second lobe, become cordate and produce the embryo.

The structure of a ripe antheridium is as usual in highly advanced ferns. There is a basal funnel-shaped cell, a wall cell and an apercular cell, all containing a few chloroplasts in the living condition. Sometimes the basal cell may not be funnel shaped but only slightly depressed. The number of sperm-mother cells vary from 18 to 37, the most common number being about 30. This is the number met within the antheridia of advanced Leptosporangiate ferns. The lesser number of mother cells is perhaps due to the lack of nourishment owing to the formation of a large number of antheridia on young prothalli.

In ripe antheridia the wall cell is so much compressed that at some places its inner wall comes in contact with the outer. With the release of pressure on the discharge of the sperms, the walls bulge inwards almost closing the

cavity. The dehiscence of the antheridium occurs by the bodily lifting of the opercular cell which is thrown out with some force.

The sperm-mother cells are round and when ejected out of the antheridium gelatinise in the water outside. After a few seconds, the coiled sperm inside riggles, at first slowly and then rapidly, ultimately emerging out of it carrying a vesicle at its tail. The sperms apparently seem capable of fertilization.

The archegonia are never developed in the life-history of the gametophyte.

The embryo formation is apogamous. A few cells just behind the notch become meristematically active dividing in all plains transverse, vertical and horizontal giving rise to a short pad-like structure (Pt. VII, Fig. 2). Each of the cells at this stage contains a single nucleus and there is no irregular fusion of the nuclei of adjacent cells as shown in a magnified photograph of such a region in one focus (Pt. VII, Fig. 3). In its interior a few cells loose contents, develop thick walls and become tracheidal cells. They possess scalariform bands which cut one another frequently forming a number of reticulations. From its surface, on the underside of the prothallus, the first leaf which is pinnate with 3 lobes makes its appearance (Pt. VIII, Figs. 5, 6). This is protected in young condition by a number of multiseriate trichomes which are 3-5 cells long with the terminal cell swollen and globular. In the meantime a root apex is visible. The stem apex is the last to be differentiated and becomes protected by multicellular hairs and flattened scales which arise from the surface of the hemispherical cushion.

After producing the embryo, the prothallus stops further growth and the cells in the notch loose their meristematic activity and become more or less elongated anteriorly.

In the genus *Pteris* apogamy is already known to occur in *Pteris cretica* studied by Farlow (1874), in 10 other varieties of *Pteris cretica*, *P. sulcata*, *P. quadri-aurita* var. *argyrea*, *P. parkerii* and *P. flabellata* by Steil (1918, 1933). *Pteris biaurita* adds another to the list of apogamous species of the genus *Pteris*.

#### Summary.

The development of the gametophyte in *Pteris biaurita* is of the *Pteris longifolia* type.

There is no typical cushion which is met with commonly in ferns behind the notch in well-developed prothalli.

Antheridia with motile sperms apparently capable of fertilization are formed but archegonia are eliminated out of the life-cycle of the gametophyte.

The dehiscence of the antheridia occurs by the bodily lifting of the opercular cell which is thrown off with some force.

The embryo formation is by apogamous bud.

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# INDEX TO TEXT-FIGURES AND PLATES.

## TEXT-FIG. 1—(From soil culture).

- a, b*—Rupture of the spores at tri-radiate mark.
- c, d*—Protonema protuberance with primary rhizoid cut off by oblique wall.

## TEXT-FIG. 2—(From culture on Knop's solution).

- a, b, c, d*—Filamentous protonema of different lengths.
- e*—Beginning of expansion of the protonema.
- f*—Protonema with terminal cell rather narrow and elongated. Most of the chloroplasts are localised near the apex.
- g*—Branched protonema.

## TEXT-FIG. 3—(From soil culture).

- a*—Spatula-shaped prothallus in one of the lateral cells of which meristematic activity is begun.
- b*—A slightly more advanced stage with a two-sided lateral cell differentiated.
- c*—Two-sided lateral cell having cut a few segments on right and left. The position of the notch established.
- d*—Lop-sided prothallus with the secondary lobe small in size.

## TEXT-FIG. 4—(From soil culture).

- A prothallus with 2 lobes in which the meristem arises rather late and is obliquely anterior. The two-sided cell is differentiated.

## TEXT-FIG. 5—(From soil culture).

- An old prothallus with meristem differentiated rather late in oblique anterior position.

## TEXT-FIG. 6—(From soil culture).

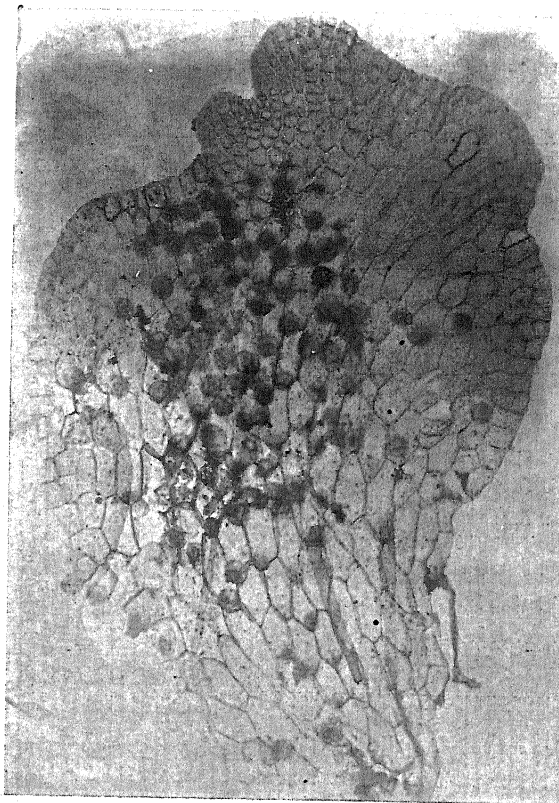
- Highly irregularly lobed prothallus. A peculiar elongated cell between two of the lobes. On either side of this cell there are small cells.

## PLATE VII.

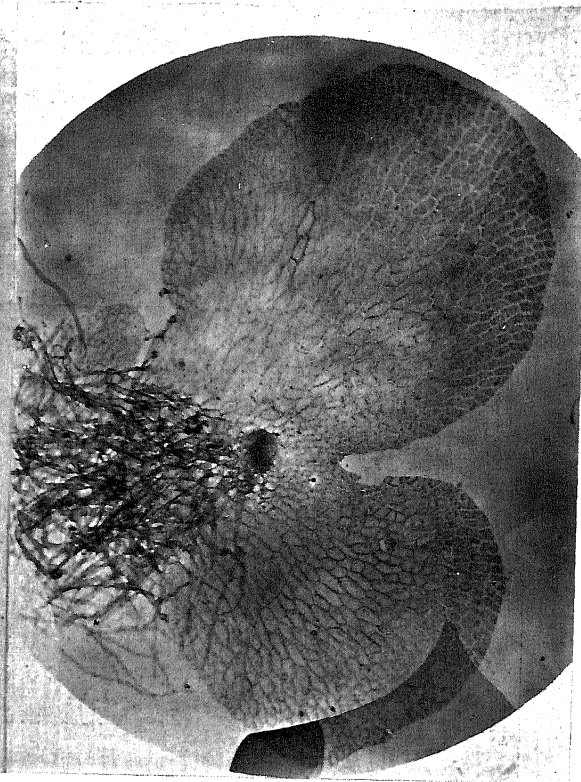
- FIG. 1.—Prothallus bearing large number of antheridia. Meristem obliquely anterior and developed rather late.
- FIG. 2.—Formation of a pad of tissue immediately behind the notch due to meristematic activity of cells in preparation to the formation of apogamous bud.
- FIG. 3.—' Pad ' region in one focus highly magnified. Each cell contains a single nucleus.
- FIG. 4.—Formation of an apogamous bud.

## PLATE VIII.

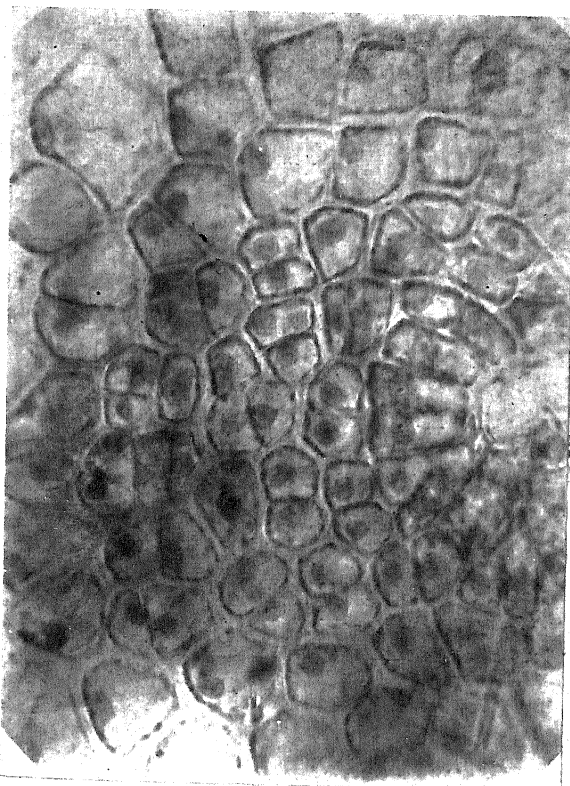
- FIG. 5.—The first leaf in process of growth.
- FIG. 6.—Later stage than 5. First leaf pinnate with 3 lobes and covered with multiseriate trichomes.



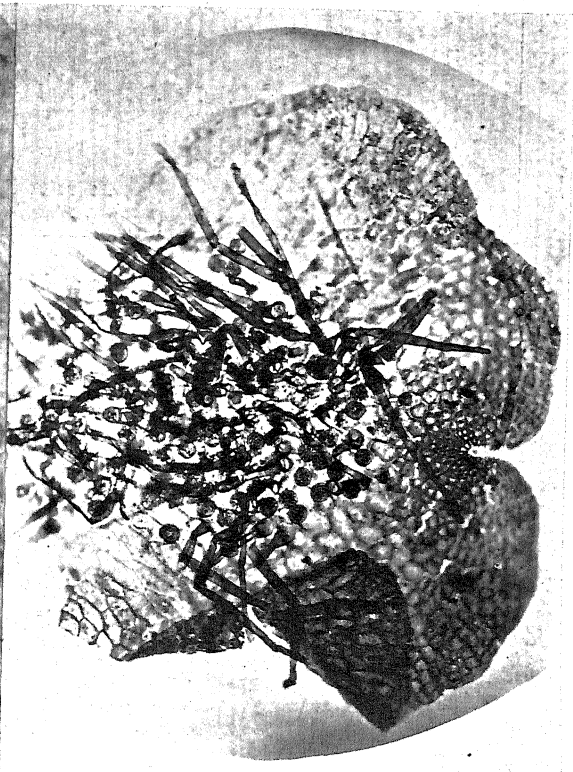
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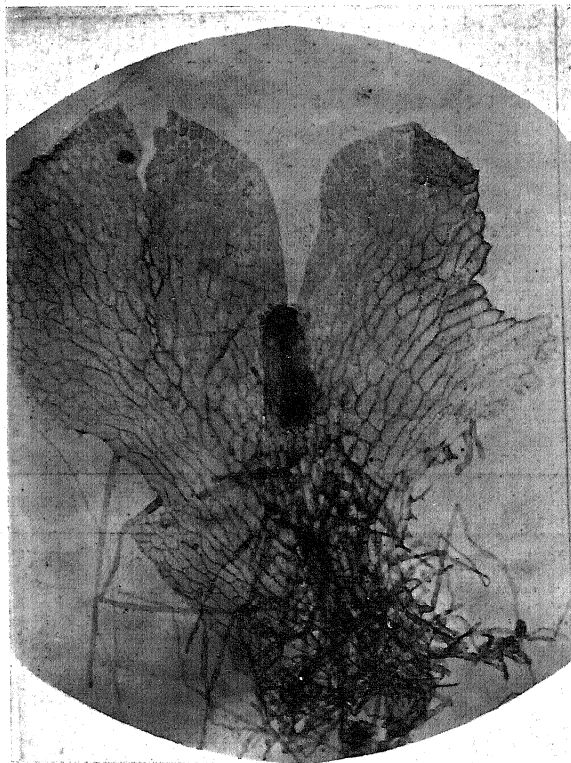
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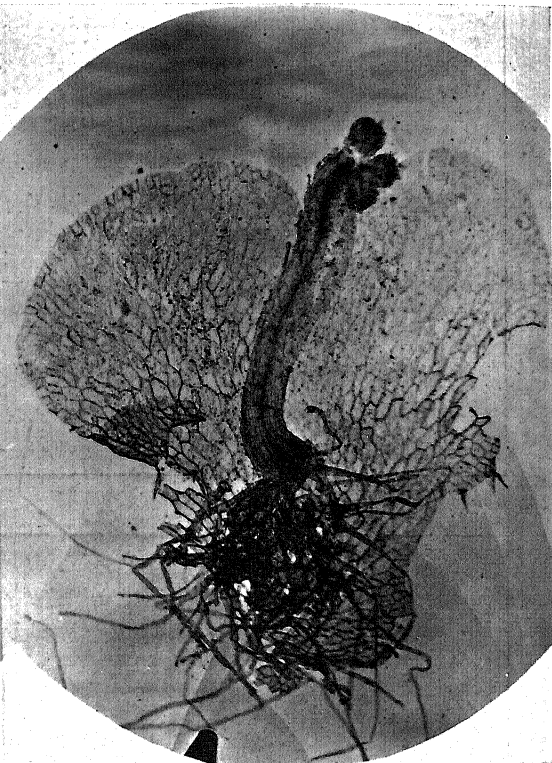
3



2



5



6

# SOME ABNORMALITIES IN THE FEMALE STROBILUS OF *GINKGO BILOBA* L.

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Received May 30, 1938.

[Communicated by Dr. H. Chaudhuri, D.Sc. (Lond.), Ph.D., D.I.C.]

A NUMBER of abnormal female strobili have been collected by the writer from a single female tree of *Ginkgo biloba*, grown in a garden at Amritsar, Punjab. This is the only tree of the species in the Punjab plain and in the absence of any male tree the seeds are never formed.

It is known that a normal female strobilus possesses two ovules at the apex each surrounded by its own collar. One of these usually aborts while the other grows to its mature size but under favourable conditions both may grow to maturity.

In the present case the tree bears strobili mostly with three ovules, one in the centre terminating the axis of the strobilus and two lateral ones, arising at about the same level, one on either side. The central ovule usually comes to maturity and the lateral ones abort (Pt. IX, Fig. 1). In some cases the central and one of the lateral ovules abort while the other lateral one develops. Rather rarely normal strobili bearing only two ovules are observed on this tree. In rare cases more than three ovules are found on a strobilus. In one instance four ovules and in another five are observed (Pt. IX, Figs. 2, 3). In both these strobili as in preceding ones only one ovule matures while the others abort. Instances of a number of ovules on a strobilus have also been recorded by Sprecher (Chamberlain)<sup>1</sup>, Fugii (quoted in 2) and Seward and Gowan.<sup>2</sup>

The ovules mature in the Punjab about the middle of July when the female gametophyte is fully developed. The normal ovules are smooth, more or less oval and the integument differentiated into the usual three layers, outer fleshy, middle stony and inner papery, completely encloses the female gametophyte. In addition to the normal ones a number of abnormal ovules are observed.

In some the lower region of the integument is smooth but the apical region is formed of irregular tumor-like swelling. The tissue here is rather loose and fluffy and is not differentiated into the usual three layers. The

top portion of these ovules is usually open so that the female gametophyte is exposed (Pt. IX, Figs. 4, 5). In one case the top one-third of the female gametophyte was exposed and consequently became green in colour. The gametophyte in some of these ovules may be more or less conical at the apex (Pt. IX, Fig. 4).

In one case two ovules were invested by a common collar instead of each having its own. One of the ovules was, however, aborted while the other fully developed (Pt. IX, Fig. 6).

A most interesting abnormality is the occurrence of 'double ovules' to the extent of 20 per cent. in the strobili collected. These unlike the normal ones, are broad and flat with a depression in the middle running vertically all over (Pt. X, Fig. 7). Each possesses a single collar. A vertical section shows that there are two chambers separated from one another by a wall of the middle stony layer of the integument and each possessing a fully developed female gametophyte (Pt. X, Fig. 8). The outer fleshy layer of the integument is continuous, the middle stony layer is also continuous but also forms a partition separating the two loculi while the inner papery layers of the two chambers are distinct.

All degrees of abortion of one of the two female gametophytes in such 'double ovules' are observed. In these cases the second chamber is always present, however poorly developed it may be, depending upon the stage of abortion of the female gametophyte within. These ovules therefore are asymmetrical possessing a large lobe within which is the fully developed gametophyte and a small lobe of variable size with the aborted gametophyte within (Pt. X, Figs. 9, 10).

Occasionally ovules with an aperture on one of the lateral sides are observed (Pt. X, Fig. 11). These in vertical section show that the chamber with aborted female gametophyte possesses an orifice communicating with the exterior (Pt. X, Fig. 12).

In rare instances 'double ovules' with both the chambers sterile are seen and then the ovules remain small in size.

*Anatomy of the Axis of a Strobilus with two Ovules each with a Short Stalk.*

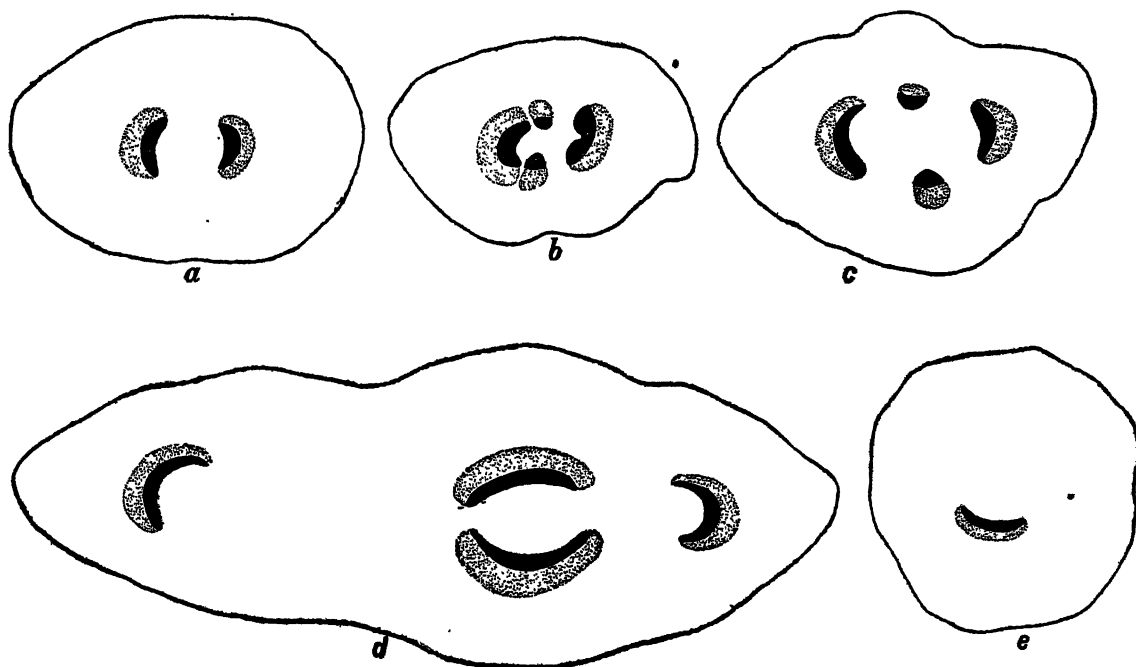
Two vascular bundles enter the strobilus axis from the stem and run through it as such. Near the apex of the strobilus each bundle enters the short stalk of each of the two ovules. The stalk thus receives only a single bundle which as it reaches the ovule expands in the form of an arc.

This observation differs from that of Seward and Gowan,<sup>2</sup> who found in a two-ovulate strobilus axis four bundles in two pairs.

*Anatomy of the Axis of Abnormal Strobili.*

*A. 3-ovulate strobilus of which the terminal ovule alone is fertile (Pt. IX, Fig. 1).*

Two vascular bundles opposite one another enter the base of the strobilus from the stem (Text-Fig. 1*a*) and proceed as such upto about  $\frac{1}{3}$  the length of the axis. From the margins of one of these, two bundles are cut off towards the inside (Text-Fig. 1*b*). These two new bundles also receive their share from the margins of the other bundle as well. Thus four bundles are formed, two lateral larger and two central smaller (Text-Fig. 1*c*). The central bundles gradually increase in size till near the place of attachment of the lateral ovules (which arise at about the same level) they become bigger than the lateral bundles (Text-Fig. 1*d*). This is because the former have to supply the fully developed terminal ovule. Each lateral bundle passes into the pedicel of the aborted ovule on its side (Text-Fig. 1*e*).



TEXT-FIG. 1.  $\times 25$

Upwards the two central bundles become continuous in the form of a ring in the collar region to which traces are supplied from all sides and the stele enters the placentrum on which the ovule is borne. The vascular ring contracts here and divides into two traces which pass through the fleshy and the stony layer into the innermost papery layer where they run along its

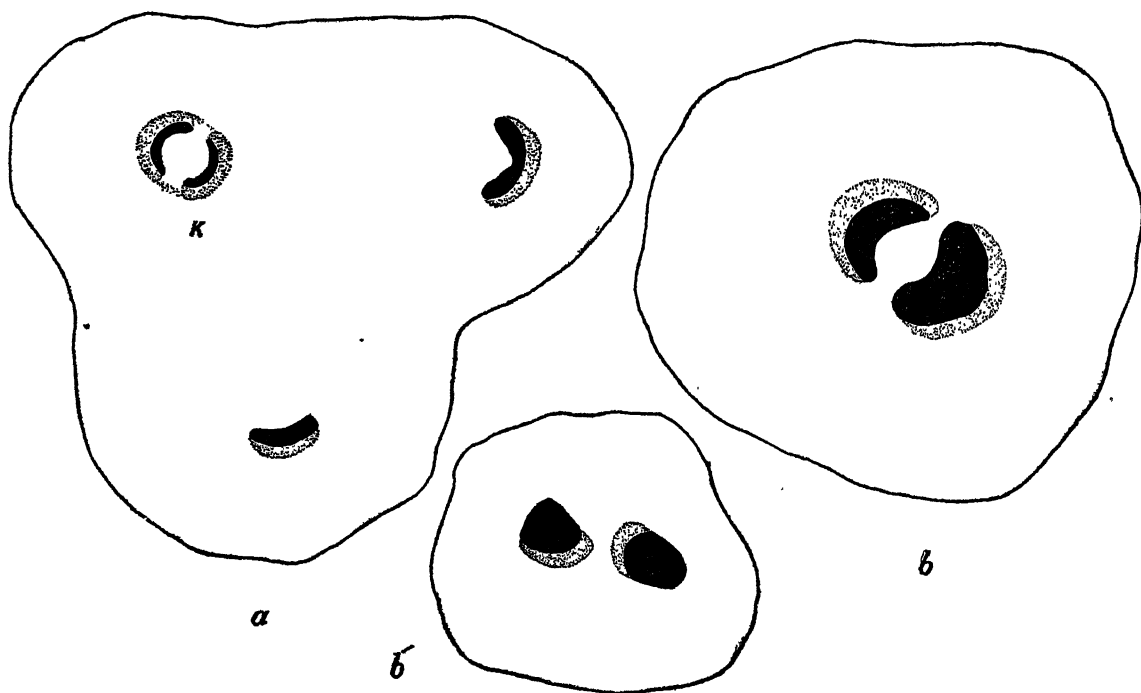
length on either side. The outermost fleshy and the middle stony layers are devoid of any vascular supply.

*B. 3-ovulate strobilus with one of the lateral ovules only fertile.*

Only two bundles enter the strobilus axis from the stem and become converted into four as described previously. The two central bundles which supply the central ovule remain small since it is aborted. Each one of the lateral bundles supplies the pedicel of the ovule on its side. In the lateral fertile ovule the single bundle passes upwards and without dividing enters the collar region. Here it forms a continuous ring and supplies the usual traces to the collar. The further course is as described for the fertile ovule in A.

*C. 4-ovulate strobilus in which the two lateral ovules are aborted and the terminal one is a "double ovule" (Pt. X, Fig. 7).*

The history of the strobilus axis is the same as in previous cases until a vascular ring with 4 bundles is formed. The two lateral ones pass into the pedicels of the lateral aborted ovules—one in each and the two central bundles enlarge and form a more or less continuous vascular ring (shown at K in Text-Fig. 2 a). Because of the presence of a 'double ovule' at the apex, the



TEXT-FIG. 2.

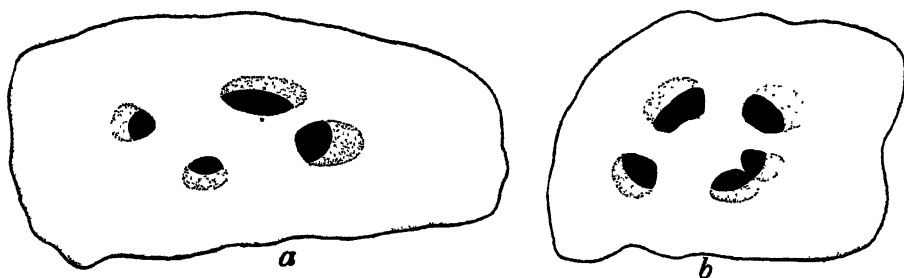
× 25

following interesting changes take place. The vascular ring splits into two vascular bundles which separately enter the placentrum through the collar to which traces are given from both. The placentrum, on which the ovule is borne, is transversely elongated and possesses two papillæ separated from one another by a depression. It gives the appearance as if it is formed of the fusion of two placenta. Each papilla receives one of the two vascular bundles from below. Here each vascular bundle divides into two in each papilla and the two pairs pass through the outer fleshy and middle stony regions to the inner papery integuments of the two chambers one pair in each chamber. Thus the papery integument of each chamber of the double ovule receives a pair of bundles as the papery integument of a normal ovule.

In the asymmetrical double ovule (Pt. X, Fig. 9) where the female gametophyte in one chamber is abortive and the chamber consequently ill-developed while the other chamber is fully developed with fully developed gametophyte, the size of the pair of vascular bundles in the two papillæ of the placentrum is naturally different (Text-Fig. 2 b, b').

*D. 5-ovulate strobilus with only one ovule fertile* (Pt. IX, Fig. 3).

The anatomy of the axis in this case differs from the others by the fact that four bundles instead of two enter its base from the stem (Text-Fig. 3a). They are arranged in the form of a ring. Higher up, one of these divides into two so that five bundles are formed—as many as there are the number of ovules (Text-Fig. 3b). *One vascular bundle enters the pedicel of each of the five ovules.* The further history of the vascular bundle entering the pedicel of the fertile ovule is the same as that in the fertile ovule described previously in B.



TEXT-FIG. 3.

× 25

### *Conclusion.*

The 'double ovule' which is of fairly common occurrence seem to have arisen by the fusion of the primordia for development of two normal ovules at the apex of the strobilus. The presence of two distinct papillæ on the elongated placentrum, the two pairs of vascular bundles, one pair in

each papilla, and the two loculi each with its separate inner papery integument and female gametophyte corroborate this view. Even externally the groove running longitudinally in the middle of the ovule is indicative of two ovules having fused together.

The anatomy of the axis of the female strobilus and that of the ovuliferous stalk in *Ginkgo biloba* appear to be variable.

Fugii found in the words of Seward and Gowan<sup>2</sup> that "A penduncle bearing several ovules is usually traversed by *as many vascular bundles as there are the ovules* : each of the bundles in the penduncle divides into two in the ovule stalks, so that each possesses two small strands similar to those in an ordinary leaf stalk". On the other hand, Seward and Gowan<sup>2</sup> find in the axis of an ovulate strobilus with three ovules, *as many pairs of bundles as there are the ovules*, and each pair in the ovuliferous stalk unites into an arc-shaped bundle.

In the present investigation it is found that usually two bundles enter the strobilus axis from the stem (excepting in the 5-ovulate strobilus when four bundles enter). The number of bundles in the strobilus *may be the same as the number of ovules it bears* (as in 2-ovulate and 5-ovulate strobili) or different (as in 3-ovulate strobilus when four bundles are found). Again the ovuliferous stalk receives only a single vascular bundle (unless it is the terminal ovule of a 3-ovulate strobilus) and that this single bundle *never divides into two in the stalk* of the ovule.

#### Summary.

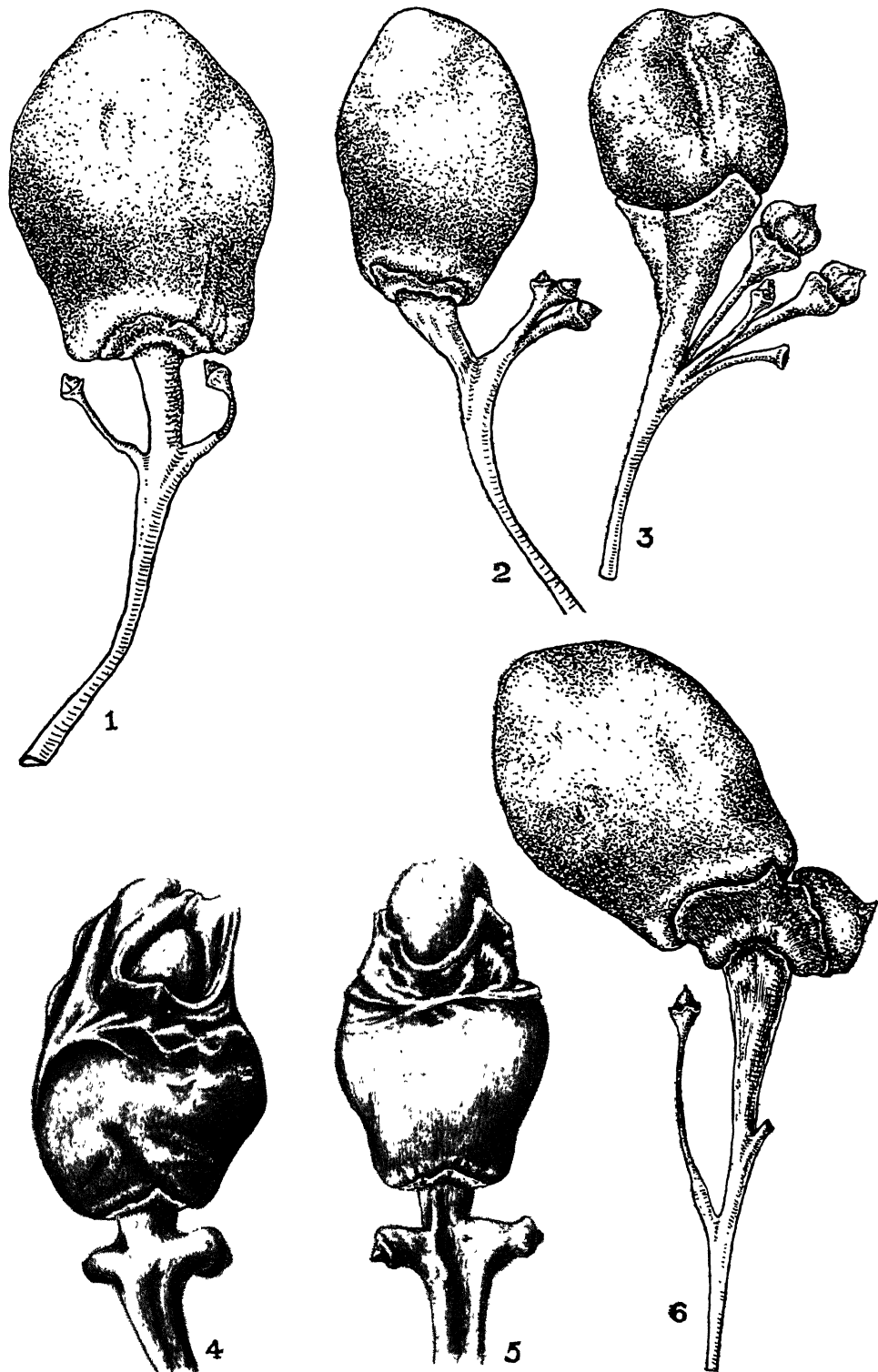
Some abnormalities in the female strobilus of *Ginkgo biloba* are recorded from the only tree growing in the Punjab plain.

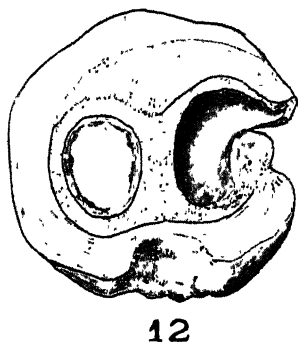
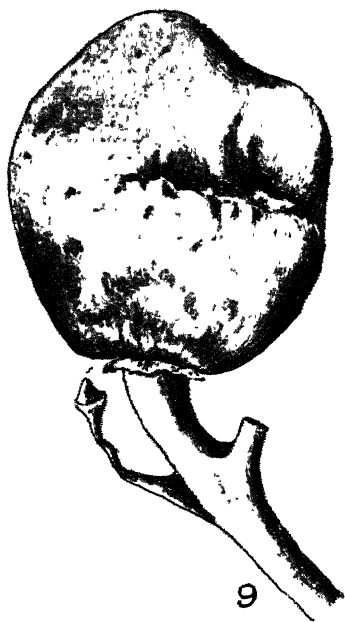
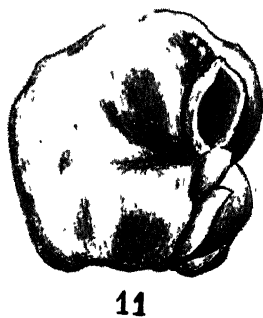
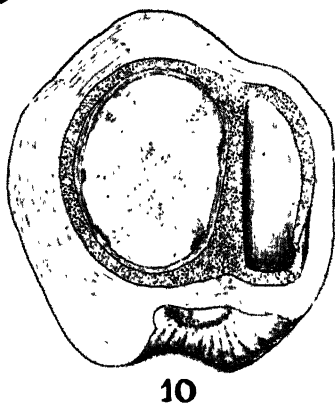
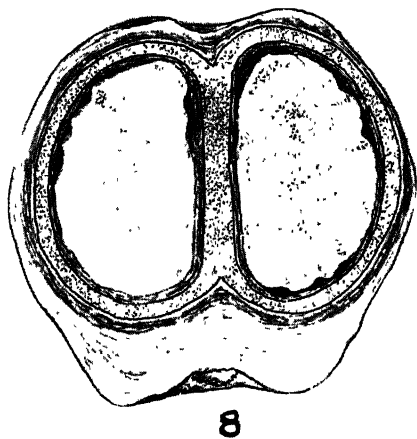
2-Ovulate normal strobili are rather rare while 3-ovulate strobili with only the terminal ovule fertile are very common on the tree. Occasionally 4-ovulate and 5-ovulate strobili are met with.

'Double ovules' formed by the fusion of the primordia of two ovules during development are met with to the extent of 20 per cent. These are flat, with a median groove. In vertical section they possess two loculi both of which may have fully developed gametophyte or only one of them. In the former case the ovules are symmetrical, in the latter asymmetrical.

Abnormal ovules having the female gametophyte exposed at the apex are frequently met with.

Anatomy of the axis of abnormal strobili has been worked out and differences from that described by previous authors pointed.





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EXPLANATION OF PLATES.

PLATE IX.

- FIGS. 1, 2, 3.—Abnormal Female Strobili with 3, 4 and 5 ovules respectively. In each case only one ovule is fertile.  $\times 2$ .
- FIGS. 4, 5.—Abnormal ovules with female gametophyte exposed at the apex.  $\times 2$ .
- FIG. 6.—Two ovules possessing a common collar. One of these is sterile.  $\times 2$ .

PLATE X.

- FIG. 7.—A Symmetrical double ovule in which female gametophyte in both loculi is developed.  $\times 2$ .
- FIG. 8.—Vertical section of the above.  $\times 2$ .
- FIG. 9.—An asymmetrical double ovule in which the female gametophyte in the smaller chamber is abortive.  $\times 2$ .
- FIG. 10.—Vertical section of the above.  $\times 2$ .
- FIG. 11.—An asymmetrical double ovule in which the loculus with abortive gametophyte communicates with the outside by an opening.  $\times 2$ .
- FIG. 12.—Vertical section of the same.  $\times 2$ .

# THE GERMINATION OF POLLEN GRAINS IN ARTIFICIAL CULTURES IN *EPHEDRA FOLIATA* BOISS AND *EPHEDRA GERARDIANA* WALL.

By P. N. MEHRA.

(Kashyap Research Laboratory, Panjab University, Lahore.)

Received June 25, 1938.

[Communicated by Dr. H. Chaudhuri, D.Sc. (Lond.), Ph.D., D.I.C.]

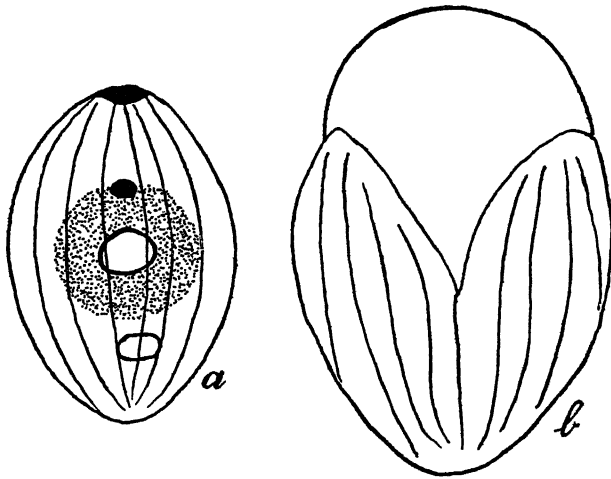
THE mature pollen grain in both the species is yellow in colour and spindle-shaped in outline. In *Ephedra foliata* it measures  $39\ \mu \times 17\ \mu$  and in *Ephedra gerardiana*  $41\ \mu \times 21\ \mu$ . The following account is true for both the species and the differences wherever met with are pointed out in the paper.

The exine is beautifully sculptured possessing ridges running longitudinally from pole to pole. At the time the pollen grain is shed it consists of remnants of two prothallial cells on one end, a small stalk nucleus usually towards the same side on the straight longitudinal axis or on the oblique axis of the grain either bordering or embedded at the periphery of a central body cell and a rather large tube nucleus towards the opposite end (Text-Fig. 1 a). While this is the usual state of affairs, departures have also been observed in the position of stalk and tube nuclei. In some cases the stalk nucleus lies on the transverse axis of the grain. Rarely the stalk and tube nuclei may be found on the same side of the grain instead of on the opposite sides.

The body cell is naked, clearly marked off from the cytoplasm of the grain by its circular or more or less elliptical outline and possesses a body nucleus in the centre. The germination of the pollen grains has been tried on the mucilage secretion that oozes out of the micropylar end of the mature ovules ready for pollination and on different strengths of Sachharose solution.

*Mucilage Secretion.*—In nature the mucilage secretion serves as a medium for the germination of pollen grains in the micropylar tube or the pollen chamber. The secretion is given out of a mature ovule in the form of thick shining drop at any time during the day but mostly during the early morning hours. If the secretion drop is removed from an ovule or if it gets dried up before the pollination is secured another oozes out on the following day and if this meets with the same fate, still another is given out after another

24 hours, although this is decidedly of a smaller size. If, however, the pollination does not occur even at this time, no more secretion is exuded. The



TEXT-FIG. 1.

ovules thus retain their power of receptivity of the pollen grains for at least 3 days.

Experiments show that it is possible to germinate the pollen grains of either species on the secretion of the other species with equal vigour as on its own secretion. It appears, therefore, that the chemical composition of the ovule secretion of the two species is similar in so far as it effects the germination of pollen grains.

The mucilage secretion is received upon a glass slide and upon this pollen grains are shed from ripe stamens. The slide is then placed in a moist chamber with saturated atmosphere. On account of its mucilaginous nature, the secretion absorbs water from the atmosphere within an hour or so and gets diluted. This is most essential for the successful germination of the pollen grains.<sup>1</sup> The pollen grains shed their exine 2-3 hours after the time they are put on the secretion. It is possible to reduce this period to

<sup>1</sup> In an experiment where the pollen grains were shed on the mucilage secretion on glass slide which was kept in a closed chamber in ordinary atmospheric temperature and humidity, the pollen grains failed to show any sign of germination even 6 hours after their being put on the medium. After 20 hours only a very few grains had ruptured their exine. When, however, a drop of pure water or 10% Sachharose solution was added, the secretion became slightly diluted and almost all the grains shed their exine and began putting out tubes. It is thus clear that dilution in strength of the secretion is very essential for germination of pollen grains. This indicates that in nature during the germination of grains upon ovules, some watery fluid must be given out for diluting the strength of the secretion.

about  $1\frac{1}{2}$  hours or less if the suitable dilution of the secretion is brought about previously to the shedding of pollen grains, by adding a drop of pure water or 10% Sachharose solution.

Various strengths of Sachharose solution 10%, 20%, 30%, 40% were tried as media for germination of pollen grains. The results in these as also in the mucilage secretion are given in Table I.

A glance at the above table shows that practically no germination takes place in 10% and 20% Sachharose solution.<sup>2</sup> Keeping the pollen grains in the media even for 54 hours does not produce any result beyond their general swelling and irregular lobing of the body nucleus which ultimately fragments into chromatin granules. The germination in 40% solution is slightly better than in 30% but the natural mucilage secretion is decidedly the most nutritious as a medium. The growth of the pollen tubes is far more vigorous in this than in Sachharose solution of any strength.

*Germination Process.*—When put in a suitable medium pollen grains absorb the nutritive medium, swell in size and assume a more or less oval appearance. The body nucleus enters the prophase stage immediately. The exine bursts by two longitudinal splits starting opposite one another from the tube nucleus end of the grain and extending on one side almost along the entire length of the grain while on the other side about half the length or a little more or a little less (Text-Fig. 1 b). This takes about 2–3 hours in the natural mucilage secretion kept in a moist chamber. The 'spindle gametophyte',<sup>3</sup> bounded on the outside by the intine, jerks out of the pollen coat which immediately undergoes torsion. The tube nucleus end of the gametophyte is invariably foremost during this liberation in both *E. foliata* and *E. gerardiana*. Thus liberated it increases to about  $1\frac{1}{2}$  to 2 of its former size in the pollen grain. Sometimes these gametophytes are under such a high state of turgescence that on their escape from the pollen coat they burst with a puncture in their wall ejecting the contents into the medium in a fine stream. If the outer medium is diluted at this stage by adding a drop of water rapid absorption takes place in the gametophyte

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<sup>2</sup> Land (4) secured germination of pollen grains in *E. trifurca* in 10% Sachharose solution but he states that the solution thickened considerably towards the end of the experiment. No definite statement can be made, therefore, as to which strength of Sachharose brought about the germination of grains since the strength gradually rose higher from 10% by the gradual evaporation of water.

<sup>3</sup> Throughout the present paper the term 'spindle gametophyte' is used for the gametophyte which is liberated out of the exine of the pollen grain and as long as it retains its spindle-shaped outline. This term differentiates this stage from the pollen grain (with exine intact) on the one hand and the gametophyte which has formed the pollen tube on the other.

TABLE I.

Time	10% Sacch.	20% Sacch.	30% Sacch.	40% Sacch.	Mucil. Secret.
3 hrs. ..	Gradual swelling of grains. No rupture of exine.	Gradual swelling of grains. No rupture of exine.	Gradual swelling of grains. No rupture of exine.	Gradual swelling of grains. No rupture of exine.	Exine in a very large number of grains ruptured. The rest of them highly turgid.
6 hrs. ..	Similar condition as above.	The same as in 10%.	Most of the grains swollen with exine intact. A few thrown out of the exine, no sign of tube formation yet.	Most of the grains swollen with exine intact. A few grains thrown out of the exine, no sign of tube formation yet.	Exine ruptured in almost all the grains. The tube formation already initiated.
17 hrs. ..	Essentially the same as above, excepting that the body nucleus in the grains considerably swollen and lobed. In a few cases the exine ruptured but no sign of tube formation.	Practically the same as in 10%.	In some cases exine still intact but mostly exine ruptured already. In some cases tube formation not even initiated but mostly tubes of smaller length put out.	The condition only slightly better than in 30%.	Vigorous growth of pollen tubes.
48 hrs. ..	The same condition as above. No tube formation in the few grains with ruptured exine.	Practically the same condition as in 10%.	Elongated tubes formed. Some grains swollen with exine still intact.	Slightly better growth than in 30%.	Most prolific growth of pollen tubes which have become highly elongated, vacuolous and hyaline.

through endosmosis resulting in the bursting of the wall and the expulsion of the contents.

At the time the exine bursts, the body nucleus is usually in the mid-prophase stage, the earlier changes having already occurred when the exine was intact. All the further changes in the body nucleus take place in the nutritive medium outside. While this is the usual behaviour, it is not invariably so. Cases have been observed when the body nucleus may have reached the late prophase or metaphase or even sometimes the anaphase while still bounded by the exine. Usually if the exine does not rupture, the further progress in the division of the body nucleus is stopped but in rare instances it may even proceed to the formation of two male nuclei which, however, in no case increase in size probably due to the lack of space. The total time taken for the complete division of the body nucleus and the organisation of two daughter nuclei is different for different media. In mucilage secretion it takes about 5 hours from the time the pollen grains are put in the medium.

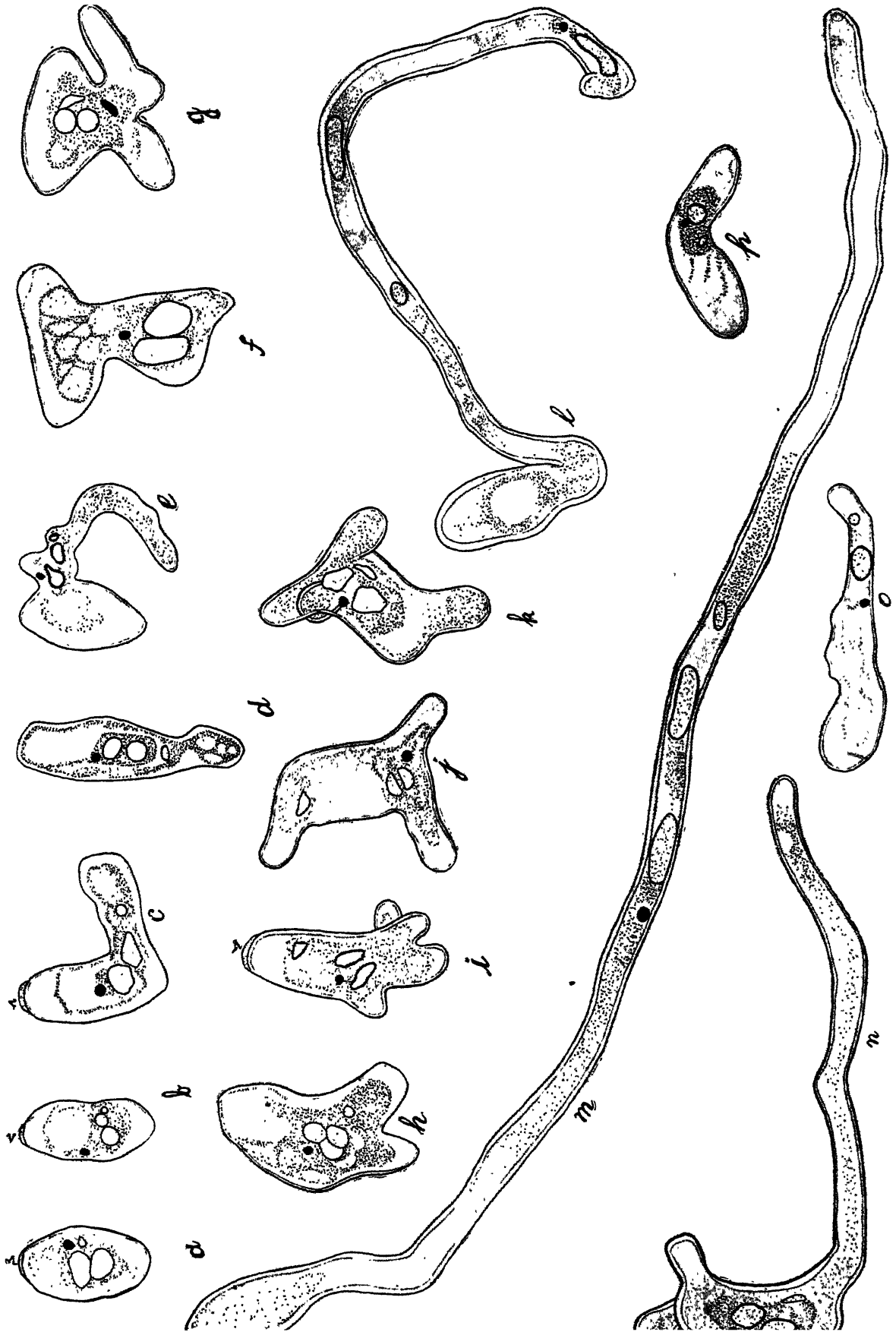
The spindle gametophytes when stained with acetic-iodine green or iron-alum hæmatoxylin sometimes show indications of evanescent vegetative cells. More often no trace of these is observable at this stage. The stalk and the tube nuclei take a greenish stain with acetic-iodine green while the male nuclei take a bluish stain. Mitochondria in the form of numerous granules of varying sizes which are refractile in the living grains are present in the cytoplasm of the body cell. They are not met with in the general cytoplasm of the grain outside the boundary of the body cell.

The male nuclei begin to increase in size while still embedded in the cytoplasm of the body cell (which retains its shape until now) within the spindle gametophyte. The two male gametes in *E. foliata* are equal in size (Text-Fig. 2 *a*). In exceptional cases they may be unequal (Text-Fig. 2 *b*). On the other hand, in *E. gerardiana* they are always unequal. The male nucleus facing the stalk nucleus is invariably bigger than the one on the opposite end (Text-Fig. 3 *a*).

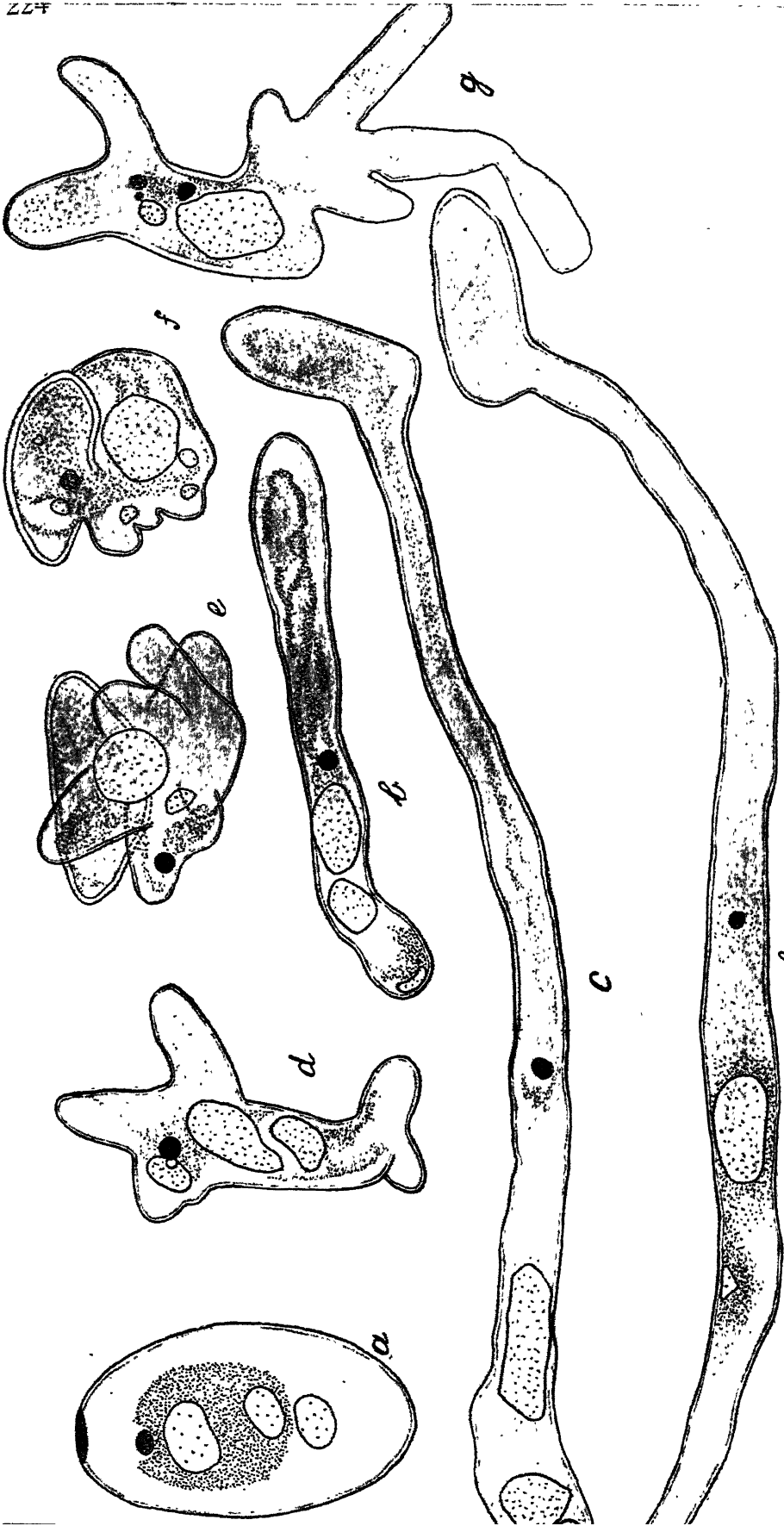
The pollen tubes begin their formation after about 6–8 hours in the mucilage secretion.<sup>4</sup> Very commonly a single tube is given out usually laterally from just near the tube nucleus end of the spindle gametophyte (Text-Figs. 2 *c*, *l* and 3 *c*, *h*). Frequently it may grow out from the mid-lateral

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<sup>4</sup> In a very crowded culture the pollen tube formation may be very much delayed or may not occur at all on account of the struggle for nutrition and space. In such a culture the male nuclei undergo a condition of senescence and develop numerous chromatin granules in their body.



TEXT-FIG. 2.



TEXT-FIG 3.

position (Text-Fig. 2 *f*) or as a direct continuation of the tube nucleus end of the spindle gametophyte (Text-Figs. 2 *d*, *m* and 3 *b*). The tubes sometimes branch immediately after their emergence (Text-Fig. 2 *g*).

Frequently the spindle gametophyte forms a swollen bladder into which the contents pass and from which a number of tubes emerge (Text-Figs. 3 *e*, *f*). Quite frequently a number of tubes from 2-6 may be given out from all sides of the spindle gametophyte in both *E. foliata* and *E. gerardiana* (Text-Figs. 2 *i*, *j*, *k* and 3 *d*, *g*). These tubes usually remain short and may commonly branch immediately after their emergence from the gametophyte. Sometimes they show a tortuous growth. Ultimately only one of these, at any position on the spindle gametophyte, develops further and the contents pass into it, the others remaining empty except for a thin lining of cytoplasm along their walls.

Land (5) figures only a single pollen tube given out from the mid-lateral position of the grain during its germination in *E. trifurca* in Sachharose solution. He makes no mention of the emergence of the pollen tube from any other position on the grain, nor does he describe anywhere the production of more than one tube or the branching of the tube.

By the time the tubes are given out, the male nuclei in the spindle gametophyte have increased considerably in size. The boundary of the body cell is soon lost, its cytoplasm becoming continuous with the cytoplasm of the grain. The nuclei embedded in the cytoplasm enter the pollen tubes where they show further growth in size. The inequality in the size of the gametes is kept up in the tube in *E. gerardiana* (Text-Fig. 3 *b*, *c*).

The sequence of the different nuclei in the pollen tube is very different even in cases where only a single tube is given out. Usually, the tube nucleus is the foremost followed in the rear by the two male nuclei which in turn are followed by the relatively smaller stalk nucleus (Text-Figs. 2 *m* and 3 *b*, *c*). In such cases in *E. gerardiana* the smaller gamete usually precedes the larger one (Text-Fig. 3 *b*, *c*). In some cases the two male nuclei are foremost followed behind by the tube nucleus and the stalk nucleus respectively. In still other cases one of the male nucleus is foremost followed by the stalk nucleus, then the other male nucleus and last of all the tube nucleus (Text-Fig. 2 *l*). When a number of tubes are put forth there is a great confusion in the behaviour of different nuclei of the gametophyte. In some cases the stalk nucleus enters one of these tubes, the tube nucleus in another and so on. Ultimately, however, all the nuclei enter the tube which shows further growth but during this irregular behaviour the sequence of the different nuclei in the tube is greatly disturbed.

These observations are different from that described by Land in *E. trifurca* (5). According to him, "the tube nucleus is foremost and one of the male nuclei passes in advance of the stalk nucleus which takes a middle position in advance of the rearmost male nucleus".

It has been possible to secure continuous growth of the pollen tubes in mucilage secretion for 48 hours when they become greatly elongated and hyaline attaining a length of 600–700  $\mu$ . The wall of these tubes glisten in living condition. The cytoplasm becomes highly vacuolous and is reduced to a thin lining along the wall of the tube. The male nuclei which by this time have become enlarged and cylindrical ultimately show the condition of senescence by the formation in their substance of a very large number of scattered chromatin granules of different sizes.

In some cases under conditions of senescence the male nuclei undergo fragmentation giving rise to 4 nuclei of different sizes. It is highly improbable that such nuclei are at all functional. If the pollen tube is kept growing for a still longer period the male nuclei ultimately degenerate and their substance is utilised for the growth of the pollen tube.

*Abnormalities.*—In *E. foliata*, the body nucleus sometimes fails to divide but enlarges and transforms itself directly into the solitary 'male gamete', which passes into the pollen tube (Text-Fig. 2 o, p). Similar instances have also been observed in *E. gerardiana* particularly in those cases where a number of pollen tubes are given out from all over the grain (Text-Fig. 3 e, g). In a well-developed gametophyte with one long pollen tube there is observed a single 'male nucleus' formed probably in a similar fashion (Text-Fig. 3 h). It is problematic if this 'male nucleus' is functional in nature.

In a few cases in *E. gerardiana* where a number of pollen tubes are initiated 5 or 6 free nuclear bodies are observed besides the single large male nucleus formed by the direct transformation of the body nucleus (Text-Fig. 3 f). It seems that these are formed by the fragmentation of the tube and stalk nuclei in response to the stimulation caused by the emergence of a number of pollen tubes. It may be pointed out that fragmentation of the tube nucleus is known to occur in some angiosperms (3).

#### Discussion.

The number of vegetative cells is differently reported in different species of *Ephedra*. Land (4) found in *E. trifurca* two vegetative cells the second of which is not separated by a wall. Strasburger (9) reports a single vegetative cell in *E. campylopodia*. Jaccard [quoted by Land (4)] did not find any vegetative cell in *E. helvetica*. He described the pollen grain in the species in a 3-nucleate condition representing in all probability the stalk, the tube and

the body nuclei. It has been found by the author that in *E. gerardiana* two prothallial cells are cut off and the nucleus of the second prothallial is separated by a wall which may early disappear or persist till the evanescence of both the vegetative cells. In *E. foliata* a similar condition is observed except that the wall of the second nucleus disappears as soon as it is formed. The species *E. gerardiana*, *E. foliata* and *E. trifurca* therefore resemble one another in the possession of two prothallial cells and form a reduction series in the male gametophyte starting with the former where there is the irregularity in the persistence of the wall separating the second vegetative nucleus to the other two species where it disappears as soon as formed. This reduction is further carried forward in *E. campylophora* where a single vegetative cell is cut off and lastly to *E. helvetica* where the vegetative cell according to Jaccard is entirely missing. This last species seems curious in having altogether eliminated the formation of vegetative cell and is worth re-investigation.

The pollen grain when shed has the generative nucleus already divided to form a stalk nucleus and a body nucleus which is organised to form a naked cell within the pollen grain. In no case among the species investigated so far is the stalk nucleus separated by a wall to form a definite stalk cell as in some lower gymnosperms.

The two male gametes are equal in size in *E. trifurca* (4), *E. altissima* (2) and as a rule in *E. foliata* described in the present paper. In *E. distachya* Berridge and Sanday (1) reported the inequality in size of the nuclei while in a later investigation Berridge (2) also reported the equality of the two nuclei which therefore indicates that in this species both conditions are met with. In *E. gerardiana* in the present investigation the two nuclei are invariably observed to be unequal in size.

A comparison of the male gametophyte of the genus *Ephedra* with that of *Welwitschia* and *Gnetum* is of some interest.

Pearson (8) found in *Welwitschia* that the pollen grains at the time of shedding contain an evanescent vegetative nucleus which is not separated by a cell wall as a definite cell, a tube nucleus and naked generative cell with a nucleus in the centre. On germination of the pollen grain on ovules, the generative cell enters the pollen tube where its nucleus divides to give rise to two unequal gametes one of which may degenerate.

The male gametophyte in *Gnetum* closely resembles that of *Welwitschia*. According to Pearson (7), Lotsy and Karsten [quoted by Pearson (8)] the pollen grain when shed contains a free vegetative nucleus, a tube nucleus and a generative nucleus which becomes organised into a generative cell at the

time of germination of the pollen grain in the micropyle of the ovule. The vegetative nucleus usually disappears at the time of pollination so that only two nuclei are observed in the pollen grain lodged on the nucellus. The generative nucleus divides and forms two gametes unequal in size. It is therefore observed that in these species of *Gnetum* there is complete absence of the stalk cell or stalk nucleus stage. The only exception seems to be in Thompson's statement (quoted by Pearson, *Gnetales*, p. 133) where he describes a tube nucleus, a stalk cell and a generative cell in the pollen grain before germination. In the face of this diversity of structure, the species described by the last author needs re-investigation.

In the living gymnosperms the stalk cell is represented in the male gametophyte either as a distinct cell separated by a wall as in Coniferales or in the form of a nucleus as in Ginkgoales. *Ephedra* retains that primitive gymnosperm level in the possession of a stalk nucleus but *Welwitschia* and *Gnetum*, where the stalk cell stage seems to be eliminated from the life-history, go above the gymnosperm level in this respect and approach a condition met with in angiosperms. This condition of advance in the latter two genera is further supported by the fact that the last vestige of the prothallial cell is left in the form of single free nucleus in contrast to the presence of one or two prothallial cells in the genus *Ephedra*.

The inequality in the size of the gametes is obviously an advance over the equal size of the gametes. This specialization is for the purpose of giving greater nourishment to one of the gametes, probably the one that brings about fertilization, at the cost of the other. *Welwitschia* and *Gnetum* are advanced in this respect in that the gametes are unequal in size. In the genus *Ephedra* we find all the stages from the equality in size of gametes in *E. trifurca*, *E. altissima*, *E. foliata* to intermediate forms like *E. distachya* where sometimes the gametes are equal and sometimes unequal in size and finally to forms like *E. gerardiana* where the inequality in size of the gametes is an established feature of the species.

#### Summary.

The pollen grain at the time of dispersal consists of a stalk nucleus on one end at the periphery of a central naked body cell and a tube nucleus on the opposite end in both *Ephedra foliata* and *Ephedra gerardiana*. Sometimes remains of two vegetative cells are observed on the stalk nucleus end of the grain.

Germination of the pollen grains has been brought about in artificial cultures on the natural mucilage secretion that oozes out of the mature ovules and on various strengths of Sachharose solution. The germination

is most vigorous on the mucilage secretion and progressively less so in 40% and 30% Sachharose while practically no germination occurs in 20% and 10% Sachharose solutions.

The germination of the pollen grain of either species occurs on the mucilage secretion of the other species with the same vigour as on its own.

The tube nucleus end of the spindle gametophytes is invariably the foremost during its liberation from the inside of the pollen coat.

The mitotic division of the body nucleus to form the two male nuclei invariably occurs within the spindle gametophyte before the emergence of the pollen tube. The entire process takes about 5 hours in the natural mucilage secretion.

There is no definite place for the emergence of the pollen tube from the pollen grain although usually it is given out laterally from near the tube nucleus end of the grain. Very commonly a number of tubes as many as six may begin emerging from a grain from all sides some of which may branch but ultimately only one develops further and the contents pass into this.

The two male nuclei in *E. foliata* are equal in size while in *E. gerardiana* they are invariably unequal.

Pollen tubes growing in mucilage secretion for about 48 hours attain a length of 600  $\mu$ –700  $\mu$  when the male gametes become senile and sometimes fragment.

In abnormal cases in both *E. foliata* and *E. gerardiana* failure in the division of the body nucleus causes it to be directly transformed to a single 'male gamete', whose functional nature is problematic.

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## INDEX TO TEXT-FIGURES.

(Stalk nucleus in all these cases is coloured black.)

TEXT-FIG. 1.—(*Ephedra foliata*)  $\times 1460$ . *a*.—Mature pollen grain; *b*.—‘Spindle gametophyte’ emerging out of the exine.

TEXT-FIG. 2.—(*Ephedra foliata*)  $\times 400$ . Remains of prothallial cells marked *v*. *a*.—‘Spindle gametophyte’ with the two male gametes equal in size; *b*.—Exceptional case of unequal gametes in the ‘Spindle gametophyte’; *c*.—Pollen tube formation laterally from near the tube nucleus end of the ‘Spindle gametophyte’; *d*.—Pollen tube formation as a direct continuation of the tube nucleus end of the ‘Spindle gametophyte’; *e*.—Similar as *c* but with tendency to branch; *f*.—Swollen bladder-like tube given out from the mid-lateral position of the ‘Spindle gametophyte’. The tube nucleus is under the male gametes; *g*.—Branching of pollen tube immediately after its emergence from mid-lateral position of the ‘Spindle gametophyte’; *h, i*.—A number of pollen tubes start their development from the tube-nucleus end of the grain; *j, k*.—Pollen tubes being given from all the sides; *l, m, n*.—Long pollen tubes developed in the mucilage secretion of the ovules. The two male gametes of the same size; *o, p*.—Abnormal cases where the body nucleus becomes itself transformed to a ‘male gamete’.

TEXT-FIG. 3.—(*Ephedra gerardiana*) All  $\times 400$  excepting ‘*a*’ which is  $\times 800$ . *a*.—‘Spindle gametophyte’ with two unequal male gametes. The one on the stalk nucleus end is invariably larger; *b*.—Pollen tube formed as direct continuation of the tube nucleus end of the grain; *c*.—Pollen tube formed laterally from near the tube nucleus end of the grain. In this as in ‘*b*’ inequality in size of gametes kept up; *d*.—Pollen tubes given from all over the ‘Spindle gametophyte’. (In *e, f, g* and *h* the body nucleus directly transformed into a single ‘male gamete’.) *e*.—A vesicle formed at the mid-lateral position of the spindle gametophyte giving out a number of tubes; *f*.—Similar as in *e*. Apparently the body nucleus and the tube nucleus having divided into a number of nuclei; *g*.—Branched pollen tube; *h*.—Fully developed pollen tube developed as in *c* but with a single ‘male gamete.’

# ON SOME GREGARINE PARASITES FROM CERTAIN POLYCHETE WORMS FROM THE ANDAMAN ISLANDS.

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AND

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IN 1930-31, during a stay of five months in the Andaman Islands, one of us (S. B. S.) examined the gregarine parasites from certain polychætes and made a large series of permanent preparations. The polychætes were collected in November, December and January from the foreshore of Ross and Aberdeen Islands, which form part of a group of islands collectively known as Port Blair. The smears were fixed with Schaudinn's fluid or Bouin's fluid, and mostly stained with Heidenhain's iron-hæmatoxylin. The hosts were kindly identified for us by Dr. B. Prashad, Director, Zoological Survey of India, and our best thanks are due to him. The parasites described in this paper are from the gut of *Lysidice collaris* Grube, the gut of *Eunice siciliensis* Grube, and the cœlome of *Amphinome rostrata* (Pallas).

The preparations were examined by both authors and preliminary observations communicated in a paper read before the Indian Science Congress session at Calcutta, in 1935. The study of the preparations has been further extended and the results embodied in the present paper.

Family STOMATOPHORIDÆ Bhatia, 1930.

The family includes seven genera, of which *Stomatophora* only is known from India.

Genus STOMATOPHORA Drzewecki, 1907

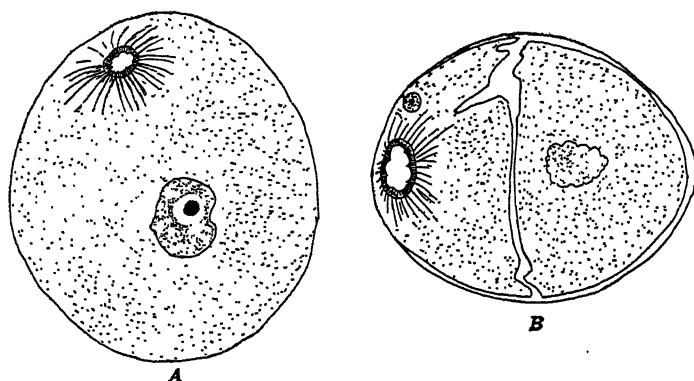
emend. Hesse, 1909, and Bhatia, 1924.

Trophozoites ovoid, spherical, cylindrical or disc-shaped. Anterior end provided with a sucker like epimeritic organ, with or without a central mucron. Sporocysts navicular, with similar non-appendiculate and truncated poles; octozoic.

All the previously known species of the genus *Stomatophora* and of other genera included in the family Stomatophoridae are parasites of earthworms belonging to the genus *Pheretima*. The species described below is the first to be recorded from a polychæte host.

*Stomatophora primitiva* sp. nov.

(Pl. XI, Fig. 1 ; Text-fig. 1.)

FIG. 1.—*Stomatophora primitiva* sp. nov.*A.*—trophozoite showing a sucker without a central mucron ; *B.*—gametocyst.*Stomatophora* sp., Bhatia and Setna, 1935, p. 312.

Trophozoite almost spherical, with a cup-like sucker near the anterior end. The sucker is not provided with a central mucron. Numerous epicytal striations radiate outwards over the body for some distance round the sucker. Nucleus is rounded with a large, spherical karyosome. Gametocysts are slightly ellipsoidal.

*Dimensions.*—Trophozoite  $222\ \mu$  in length by  $203.5\ \mu$  in width. Nucleus is  $44.4\ \mu$  by  $37\ \mu$ , and the karyosome is  $14.8\ \mu$  in diameter. Gametocyst measures  $222\ \mu$  by  $203.5\ \mu$ .

*Habitat.*—Intestine of *Eunice siciliensis* Grube: taken off Port Blair, Andaman Islands.

*Discussion.*—After discussing the morphology of the sucker in all the three species of *Stomatophora* known till then, Bhatia (1924) came to the conclusion "that unlike the epimerite of polycystid gregarines, the sucker is not present in the earlier stages of development in any of the species of *Stomatophora*; that during the growth of the trophozoite a simple cup-like sucker comes to be developed at first, by a flattening and inpushing taking place at the anterior end of the body, the median projection or mucron being thus carried to the bottom of the cup-like depression. This process would seem to cause the epicytal striations to appear first on the surface of the sucker extending from the central mucron to certain definite points on the circular border of the aperture, and later extend beyond over smaller or larger portion of the body." The epimerite of *Stomatophora* is capable of

undergoing alterations in form during the life of the parasite and is not abandoned inside a cell of the host as it is in *Cephalina*.

The species now under examination recalls in its structure *S. simplex* Bhatia, 1924, from which it differs in the absence of a central mucron in the sucker and epicytal striations radiating from it. Under the oil-immersion lens the sucker is seen to be surrounded by a clear area, and the surrounding epicytal striations appear to run into each other and interlace. In one specimen (Fig. 1, B), the sucker was seen to be retained in one of the gametocytes inside a gametocyst. The gametocyte also showed a small rounded nucleus. The other gametocyte contained a distinctly larger nucleus but did not show any sucker.

The occurrence of a mucron with epicytal striations inside the sucker have hitherto been regarded as a feature characterising the genus. In our estimation, in the more primitive forms, such as the one now under examination, there could be a simple sucker without a mucron, and we would rather amend the definition of the genus so as to include forms with suckers with or without a central mucron, than refer the new species to a distinct genus.

Family LECUDINIDÆ Kamm, 1922

emend. Reichenow, 1929.

The family originally included the genus *Lecudina* Mingaz., but Reichenow (1929) emended it so as to include *Ancora* Labbé and *Polyrhabdina* Mingaz., and has also (1932) placed *Hentschelia* Mack. and Ray and *Lecythion* Mack. and Ray in this family.

Genus LECUDINA Mingazzini, 1891.

Body cylindrical, or ovoid, non-septate; cytoplasm of the anterior part of the body usually distinctly marked off from the rest by being more finely granular. Epimerite caducous or invaginable, able to assume in any particular species only a variety of well-determined forms. Sporocysts oval, with a thickening at one pole. Intestinal parasites of polychætes.

*Lecudina eunicæ* sp. nov.

(Text-fig. 2.)

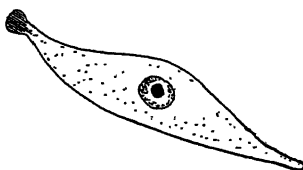


FIG. 2.—*Lecudina eunicæ* sp. nov.

Trophozoite elongate oval, widest in the middle narrowing at both ends, bluntly pointed anteriorly, and pointed posteriorly. Epimerite a knob-like

structure, with its cytoplasm differentiated from the rest of the body. Nucleus large, subspherical, with a single karyosome.

*Dimensions*.—Length of the trophozoite  $475\ \mu$ , maximum width  $94.5\ \mu$ ; epimerite  $14\ \mu$  in length; nucleus  $38.5\ \mu$  by  $28\ \mu$ .

*Habitat*.—Intestine of *Eunice siciliensis* Grube: taken off Port Blair, Andaman Islands.

*Discussion*.—The parasite somewhat resembles *L. elongata* (Mingazzini) in the differentiation of its anterior end. According to Mingazzini (1891) *L. elongata* possesses a small spherical button-like epimerite, but according to Reichenow (1932) there is no epimerite and the parasite attaches itself to the epithelial cells of the host by its differentiated anterior portion in a sucker-like manner. The present species however markedly differs from *L. elongata* in the form of the trophozoite and the structure of its nucleus.

*Lecudina lysidica* sp. nov.

(Text-fig. 3.)

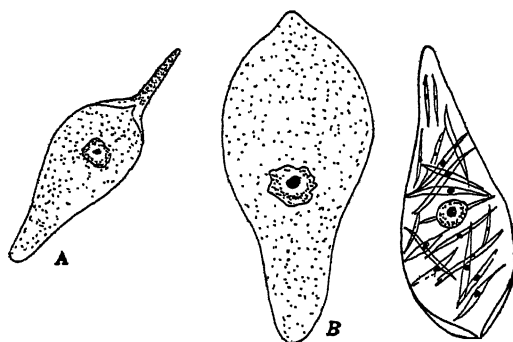


FIG. 3.—*Lecudina lysidica* sp. nov.

A.—cephalont; B.—sporont; C.—sporont containing numerous elongated, spindle-shaped algæ.

Trophozoites oval, broadly rounded anteriorly and narrower and rounded off posteriorly, broadest in the anterior third of its body. Cytoplasm does not show any differentiation in the anterior portion of the body. Epimerite is in the form of a long, conical trunk. Nucleus is spherical, oval, or irregularly quadrilateral, with the large central karyosome. Sporonts are much larger in size, without an epimerite, but show a triangular area slightly raised from the anterior end of the body.

*Dimensions*.—Cephalonts with a total length of  $71.9$  to  $185\ \mu$ , maximum width  $14.8$  to  $62.9\ \mu$ , length of epimerite  $11.1$  to  $37\ \mu$ , and nucleus  $14.8\ \mu$  by  $10\ \mu$ . Sporonts can reach a length of  $370\ \mu$  and a maximum width of  $155.4\ \mu$ .

*Habitat*.—Intestine of *Lysidice collaris* Grube: taken off Port Blair, Andaman Islands.

*Discussion*.—The parasite invites comparison with *L. aphrodite* (Lank.), but differs in its much smaller size, in the nuclear structure, and also in the epimerite not being marked with successive constrictions. The nucleus varies considerably in form and position. It may be spherical, oval or irregularly quadrilateral in form, and may be situated in the anterior, middle, or posterior part of the body, but it always presents the same structure. The central karyosome is surrounded by a clear area, and the chomatin particles are arranged in a peripheral zone within the nuclear membrane.

The sporonts frequently and the cephalonts occasionally, are seen to be full of elongated spindle-shaped bodies which are probably symbiotic algæ. These measure up to  $74\ \mu$  by  $3.7\ \mu$ , and each contains a definite central nucleus.

Family GREGARINIDÆ Labbé, 1899.

The family includes many genera of septate gregarines which show extracellular development. Epimerite is simple, symmetrical. Sporonts are solitary or in associations up to 12. Gametocysts dehisce by sporoducts or simple rupture. Sporocysts are oval or spindle-shaped.

Most of the genera belonging to this family occur as intestinal parasites of insects, only two being known from crustaceans. Genus *Ulivina* Mingazzini from polychætes has also been provisionally placed in this family by Reichenow, though nothing is known about the sporocysts in that genus. Two species belonging to new genera described below may also be provisionally included in this family till more is known about them.

Genus ULIVINA Mingazzini, 1891.

Young trophozoites with simple papillate epimerite. Sporonts free, without epimerite. Not forming syzygies. Sporocysts not known. Intestinal parasites of polychætes.

The genus *Ulivina* was based on *U. elliptica* Mingazzini, 1891, and among the characters mentioned were "external membrane forms a continuous sac round the animal and "protomerite the more dense". Neither of these characters seem to be now insisted upon. Porter (1899) described an unnamed septate gregarine from *Rhynchobolus*, but Crawley (1903) thinking that the part of the animal which Porter took to be protomerite plus epimerite was only the epimerite and the gregarine was a dicystid form, named it as *Doliocystis rhynchoboli*. Kamm (1922) referred it to *Ulivina* and placed it as a second species in that genus. Reichenow (1932, 1935) has given an amended definition of the genus and considered *Sycia inopinata* Léger,

1892, as identical with *U. elliptica* Mingazzini. It is stated as a generic character that the epimerite is surrounded at its base by a ring-like thickening. This is based on Léger's description of *S. inopinata* and not Mingazzini's description of *U. elliptica*, as the latter does not describe or figure the epimerite. According to Kamm, Saint-Joseph (1907) described *U. elliptica* with a simple, small, papillate epimerite. The ring-like thickening at the base of the epimerite is also not possessed by *U. rhynchoboli* (Crawley) or by the species described below. In our opinion *Sycia* Léger should be regarded as distinct from *Ulivina* Mingazzini. The former would include *S. inopinata* which possesses large knob-like epimerite with a thickened ring round its base and the latter would include *U. elliptica*, *U. rhynchoboli* and *U. eunicæ*, all possessing a simple papilla-like epimerite.

*Ulivina eunicæ* sp. nov.

(Pl. XI, Fig. 2 ; Text-fig. 4.)

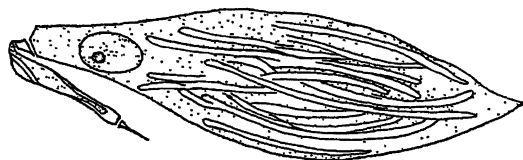


FIG. 4.—*Ulivina eunicæ* sp. nov.

Trophozoites elliptical, with the protomerite drawn out like a neck and curved backwards. Epimerite slender, wider at the base and tapering into a fine needle. The deutomerite is full of inclusions. Length of protomerite : total length as 1 : 4.3 ; width of protomerite : width of deutomerite as 1 : 6 : 3. Nucleus is oval and situated in the anterior narrow part of the deutomerite. Sporocysts not known.

*Dimensions*.—Cephalont  $340.4 \mu$  in length ; epimerite  $14.8 \mu$  in length ; protomerite  $77.7 \mu$  in length and  $11.1 \mu$  in maximum width ; deutomerite  $247.9 \mu$  in length and  $70.3 \mu$  in maximum width.

*Habitat*.—Intestine of *Eunice siciliensis* Grube : taken off Port Blair, Andaman Islands.

*Discussion*.—The parasite resembles closely *U. rhynchoboli* (Crawley), but the cytoplasm in the protomerite is not more dense than in the deutomerite, and the nucleus is oval and not spherical as in that species. Also there is no external membrane continuous round the animal.

Genus DEUTEROMERA gen. nov.

Sporonts solitary. Epimerite subconical with a cup-shaped apex. Protomerite and deutomerite showing incomplete secondary segmentation.

*Deuteromera cleava* sp. nov.

(Pl. XI, Fig. 3 ; Text-fig. 5.)

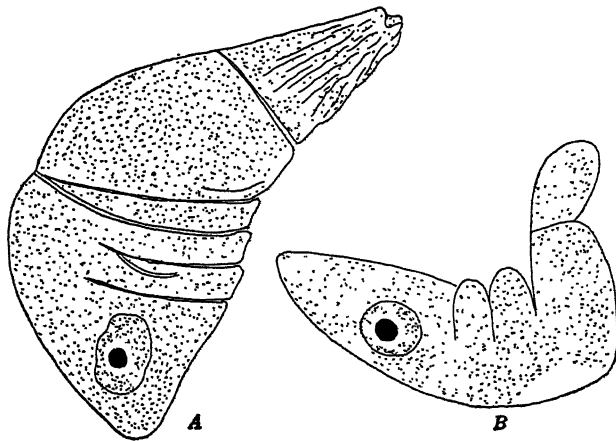


FIG. 5.—*Deuteromera cleava* g. et. sp. nov.

A.—cephalont ; B.—sporont.

Body elongate oval, distinctly septate. Epimerite simple, elongated, subconical, marked with distinct epicytal striations and with its apex somewhat cup-shaped. Protomerite not more densely granular than the deutomerite, broadest at its base, width usually greater than its length, showing a somewhat obliquely running furrow. Protomerite along with the epimerite bent at an angle on the deutomerite. Deutomerite widest anteriorly, and narrower and rounded posteriorly, its maximum width somewhat greater than the length. Deutomerite is marked across by two incomplete septa running inwards from one side. Length of the protomerite : total length as 1:3.8 ; width of the protomerite : width of the deutomerite as 1:1.3. Nucleus large, oval, situated near the posterior end. It contains a single large, spherical, excentrically placed karyosome which is surrounded by a narrow clear area, outside which are densely packed chromatin granules. Sporont solitary, proportionately narrower and much more elongated than the cephalont, may be bent upon itself and the deutomerite may show incomplete septa. In the sporont the nucleus is oval or spherical and is similar in structure to that in the cephalont. Cyst and spore-formation not known.

*Dimensions*.—Cephalont reaches upto  $402.5\mu$  in total length ; epimerite  $105\mu$  in length and  $77\mu$  in maximum width ; protomerite  $105\mu$  in length and  $147\mu$  in maximum width ; deutomerite  $192.5\mu$  in length and  $203\mu$  in maximum width ; nucleus  $52.5\mu$  by  $45.5\mu$ . Sporont reaches up to

420  $\mu$  in total length and 105  $\mu$  in maximum width, and its nucleus measures 42  $\mu$  in diameter.

*Habitat*.—Intestine of *Eunice siciliensis* Grube: taken off Port Blair, Andaman Islands.

*Discussion*.—The chief characteristic of the new form is the secondary segmentation of the protomerite as well as the deutomerite. The furrow in the region of the protomerite and the incomplete septa and a furrow in the deutomerite strongly recall the more complete segmentation of the posterior part of the deutomerite in *Metamera schubergi* Duke, 1910, known from the intestine of certain leeches, and the segmentation of the deutomerite in *Tæniocystis mira* Léger, 1906, from the intestine of a dipteran larva, and of both protomerite and deutomerite in *T. legeri* Cognetti, 1911, from the coelome of an oligochæte. The present species however shows important differences from all these species. The systematic position of *Metamera* is unknown, and *Tæniocystis* is placed in the family Actinocephalidæ. Owing to the simple character of the epimerite *Deuteromera* would seem to belong to the family Gregarinidæ, but it cannot be placed with certainty till more is known about its life-history.

Genus *CONTORTIOCORPA* Bhatia and Setna, 1935.

Sporonts solitary. Body spirally twisted upon itself.

*Contortiocarpa prashadi* Bhatia and Setna, 1935.

(Pl. XI, Fig. 4, Text-fig. 6.)

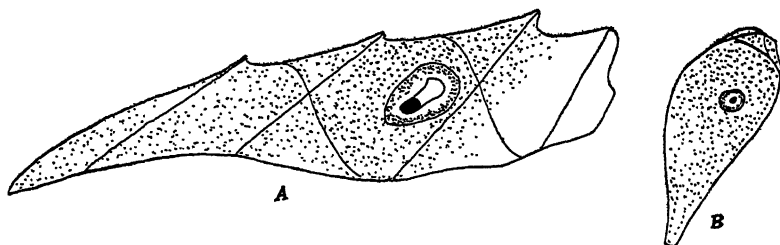


FIG. 6.—*Contortiocarpa prashadi* Bh. & Set.

A.—sporont showing the twisted body; B.—an individual not showing the twist, and with the retracted epimerite.

*Contortiocarpa prashadi*, Bhatia and Setna, 1935, p. 312.

Sporont solitary. Body elongate oval, broadest at about one-third the length of the body from the anterior end, rapidly narrowing behind the middle and drawn out posteriorly into a narrow rounded tip. The body is generally twisted upon itself, presenting a number of turns of a spiral running round it, and a number of marginal projections where the spiral is turning round to

run over the other surface. Nucleus is oval or spherical, situated usually in the anterior half of the body, and contains a single karyosome. Some individuals are also met with in an untwisted or partially twisted condition.

*Dimensions*.—Length of the body in a twisted individual  $318.5\ \mu$ , maximum width  $87.5\ \mu$ ; nucleus  $37.5\ \mu$  in diameter. A partially twisted individual measured  $398.4\ \mu$ , in length and  $170.2\ \mu$  in its maximum width.

*Habitat*.—Intestine of *Eunice siciliensis* Grube: taken off Port Blair, Andaman Islands.

*Discussion*.—In addition to the typical twisted individuals there are in the preparations a number of partly twisted or not twisted individuals which are believed to be of the same species. One such individual shows an indication of a narrow protomerite and a bluntly conical epimerite in a retracted condition. Typical cephalonts have not been met with, nor have we come across any cysts or spores which may be definitely assigned to this species.

Family SELENIDIIDÆ Brasil, 1907.

The family includes a single genus.

Genus *Selenidium* Giard, 1884

emend. Brasil, 1907, and Ray, 1930.

Trophozoites elongate, vermiform, very narrow and cylindrical or wider and more or less flattened, with longitudinal myonemes along the entire length of the body. The anterior end of the body is provided with a small knob-like organ of fixation, and usually contains characteristic chromatic bodies. Schizogony where known, takes place during the intracellular condition of the parasite. Gametocyst where known, contains many oöcysts, each containing either four or eight sporozoites.

*Remarks*.—Ray (1930) has re-studied several imperfectly known species of this genus and described several new ones. He has shown that intracellular schizogony does not normally occur in the majority of species studied by him, and is in fact known to occur in two species only. The various species behave very differently from one another, in the length of time they pass within the epithelial cells of their host. He lays stress on the occurrence in all the species examined and at all stages of their development, of characteristic chromatic bodies at the anterior end of the animal. These are usually thread-like, sometimes club-shaped, but always of a definite type and length in any particular species, and usually pretty constant in number. Very little is known about sporogony also in the species of this genus. Gametocytes were seen by Caullery and Mesnil in two species, in association in the gut and according to them the attachment was by their anterior ends. Ray

found that in the species examined by him, the associates become attached by their posterior ends. Spores had been previously seen in one species only, and were known to contain four sporozoites. Ray found gametocysts and spores in two species, and the spore contained four sporozoites in one species and eight in the other. In view of the above-mentioned considerations, he came to the conclusion that the genus requires drastic revision and will probably have to be dismembered. He also supports the view previously held by Mesnil (1899) and Keilin (1923) that the Schizogregarinaria are a heterogeneous and artificial group, and that certain genera now placed therein would one day be transferred to the Eugregarinaria.

*SELENIDIUM AMPHINOMI* sp. nov.

(Text-fig. 7.)

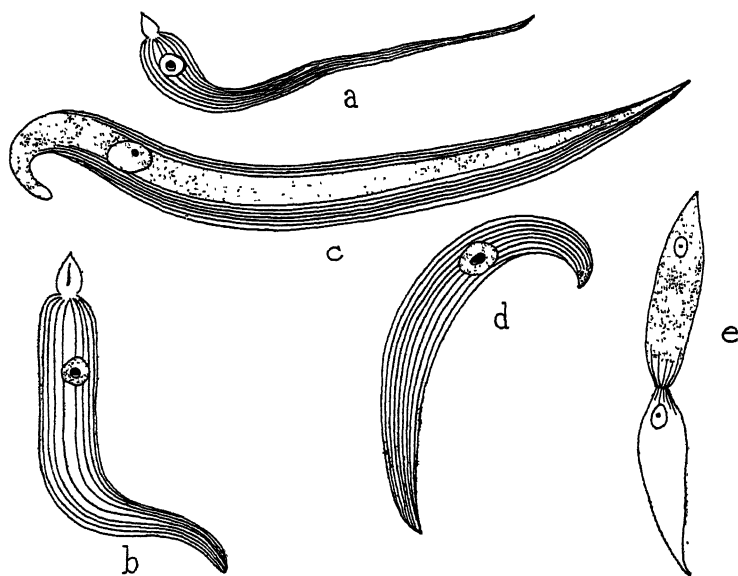


FIG. 7.—*Selenidium amphinomi* sp. nov.

a.—Young trophozoite ; b.—older trophozoite ; c, d.—gametocytes ;  
e.—association of two individuals by their anterior ends.

*Nematocystis* sp., Bhatia and Setna, 1935, p. 312.

Trophozoites elongate, vermiform, wider anteriorly and narrower and tapering posteriorly. Anterior end provided with a conical knob-like projection which usually does not show the chromatic bodies. The body is more or less circular in transverse section and the surface is marked by about sixteen longitudinal striations. Nucleus spherical or sub-spherical, situated in the broader anterior part of the body, with a large central karyosome.

Larger individuals, apparently gametocytes, show a somewhat flattened body, with the anterior end drawn in, but still differing in its appearance from the rest of the body, and the posterior end is wider than in the younger trophozoites and narrows gradually to a point. The myoneme striations are more numerous and may be about twenty in number. The nucleus in these specimens is subspherical, or oval and placed with its long axis along the length of the body. Association of the individuals is by their anterior ends. Gametocysts are sub-spherical or oval. Spore-formation not observed.

*Dimensions.*—Trophozoites  $126.8 \mu$  to  $253.6 \mu$  in length, with a maximum width ranging from  $10 \mu$  to  $25.3 \mu$ ; epimerite  $4.7 \mu$  to  $9.5 \mu$  in length and about the same in its width. Nucleus  $9.5 \mu$  in diameter, or when oval  $10 \mu$  by  $6 \mu$ . Gametocysts are  $47.13 \mu$  to  $81.69 \mu$  in length by  $45.55 \mu$  to  $62.84 \mu$  in width.

*Habitat.*—Coelome of *Amphinome rostrata* (Pallas): taken off Port Blair, Andaman Islands.

*Discussion.*—We have not been able to find any evidence of schizogony in this species, but we cannot speak with any certainty on this point, as our observations are based on the examination of smears only. The chromatic threads or bodies, on the occurrence of which during all stages, Ray (1930) lays so much stress, are not to be found in many of the specimens. One specimen, however, shows a single deeply stained thread, and two other specimens show a varying number of chromatic dot-like bodies. The association of the individuals is by their anterior ends (Fig. 7e) as was described by Caullery and Mesnil (1899) in *Selenidium echinatum*, and contrary to what has been found by Ray (1930) in other species. Although we have come across a few gametocysts, we have not found any containing ripe oöcysts.

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## EXPLANATION OF PLATE XI.

FIG. 1.—Microphotograph of *Stomatophora primitiva* sp. nov.

FIG. 2.—Microphotograph of *Ulivina eunicæ* sp. nov.

FIG. 3.—Microphotograph of *Deuteromera cleava* g. et sp. nov.

FIG. 4.—Microphotograph of *Contortiocorpa prashadi* g. et sp. nov.

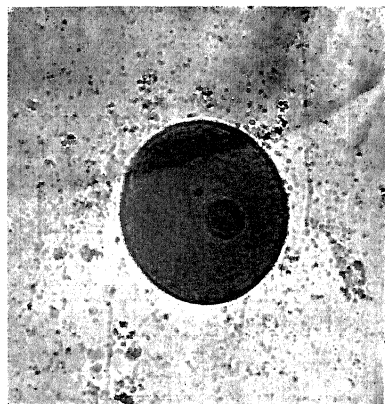


FIG. 1.

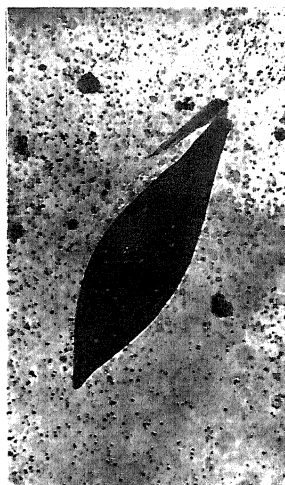


FIG. 2.

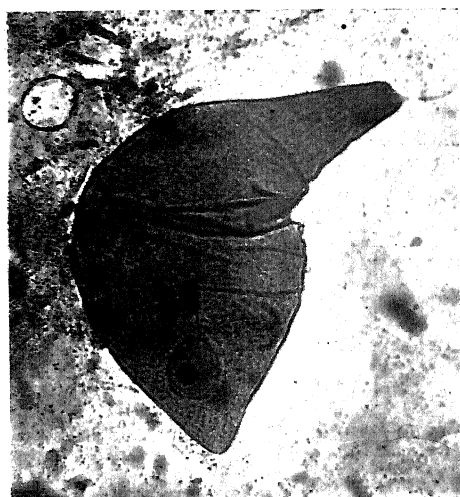


FIG. 3.



FIG. 4.



# PROCEDURE FOR DETERMINING THE NATURE OF THE DEGRADATION PRODUCTS DURING PROTEOLYSIS.

BY V. RANGANATHAN AND B. N. SASTRI.

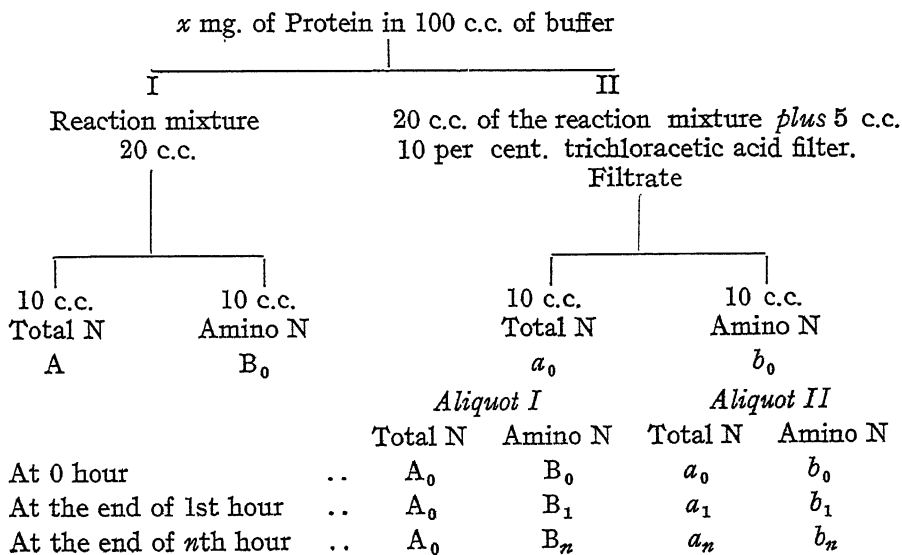
(From the Department of Biochemistry, Indian Institute of Science, Bangalore.)

Received August 30, 1938.

THE employment of specific enzymes as tools in the stepwise degradation of proteins has yielded valuable information on the chemistry of proteins. In the study of proteolysis, it is essential in the first instance, to determine the complexity of the resulting peptides, and later, to understand the nature of the constituent amino-acids. Precise information on the mode of arrangement of the amino-acids in a given peptide, can be obtained by the method of Bergman (1936), according to which the peptide chain can be progressively degraded, one amino-acid after another being detached and identified. For many purposes however, an idea of the nature of amino-acid make-up of the peptide units is of interest and in what follows, a procedure for obtaining such information is described.

*Procedure.*—A weighed quantity of the protein was dispersed in a suitable buffer and incubated with the enzyme. At noted intervals, two aliquots (20 c.c.) were withdrawn from the reaction mixture. To one, enough trichloroacetic acid (10 per cent. strength) was added to precipitate the proteins and filtered; the total and amino nitrogens of the filtrate and also of the other aliquots were determined by the usual methods.

Let the values be represented by  $A_0$ ,  $B_0$ ,  $a_0$ ,  $b_0$ , as shown below.



$A_0$  represents the total N in the reaction mixture and it will have a constant value throughout. The other factors increase progressively with proteolysis upto a constant value.

Let  $C_1$  represent  $\frac{A_0}{B_0}, \frac{A_0}{B_1}, \dots, \frac{A_0}{B_n}$ .

Factor  $C_1$  gives an idea of the complexity of the protein degradation products.

Let  $C_2 = \frac{A_0 - a_0}{B_0 - b_0}, \frac{A_0 - a_1}{B_1 - b_1}, \dots, \frac{A_0 - a_n}{B_n - b_n}$ . This will give the complexity of the protein degradation products precipitated by trichloroacetic acid that are released from the protein molecule. This will be different from  $C_1$  in the sense that factor  $C_1$  will represent the sum total of the effect due to enzyme action whereas factor  $C_2$  will represent the complexity of the degradation products precipitated by trichloroacetic acid.

Let  $C_3$  represent 
$$\frac{(A_0 - a_{n-1}) - (A_0 - a_n)}{(B_n - b_n) - (B_{n-1} - b_{n-1})} \quad (1)$$

If  $(B_n - b_n) - (B_{n-1} - b_{n-1}) = K (a_n - a_{n-1}) \quad (2)$

$C_3 = 1/K. \quad (3)$

From (2), it is possible to calculate the value of  $K$  at the  $n$ th hour, all the factors in the equation being known.  $C_3$  will give the complexity of the protein degradation products precipitated by trichloroacetic acid during the interval of time between the  $(n-1)$ th and  $n$ th hours of hydrolysis.

Coming to simpler degradation products not precipitated by trichloroacetic acid,

Let  $C_4$  represent  $\frac{a_0}{b_0}, \frac{a_1}{b_1}, \dots, \frac{a_n}{b_n}$ .  $C_4$  will give us an idea of the nature of the simpler products released from the protein molecule. This value progressively decreases.

Let  $C_5$  represent  $\frac{a_n - a_{n-1}}{b_n - b_{n-1}}$ . This will give us the complexity of the products released during a particular interval of time between  $(n-1)$ th and  $n$ th hours. A correction has to be applied to this factor for reasons given below, and such a factor is designated  $C_6$ .

$a_n - a_{n-1}$  is the amount of total N released during a particular interval of time between  $(n-1)$ th and  $n$ th hours of hydrolysis from the protein molecule as a result of enzyme digestion.  $b_n - b_{n-1}$  does not represent the amount of amino-nitrogen released during a particular interval of time from  $n$ th to  $(n-1)$ th hour.  $b_n - b_{n-1}$  is actually composed of two factors; one is the amino-nitrogen due to  $a_n - a_{n-1}$  represented as  $X (a_n - a_{n-1})$  that comes into the non-protein fraction as a result of enzyme digestion

during the interval of time from the  $n$ th to  $n - 1$ th hour, and the other  $Y$  ( $a_{n-1}$ ), representing the increase in the amount of amino-nitrogen of  $a_{n-1}$  at the  $n$ th hour.

$$b_n - b_{n-1} = X(a_n - a_{n-1}) + Y \cdot a_{n-1}.$$

The quantities  $X$  and  $Y$  vary and can be determined in the following manner.

Non-Protein Nitrogen			
	Total N	Amino N (further hydrolysis)	Amino N
0 hr.	$a_0$		$b_0$
1 hr.	$a_1$	$d_0$	$b_1$
2 hrs.	$a_2$	$d_1$	$b_2$
3 hrs.	$a_3$	$d_2$	$b_3$

At the end of the first hour, a certain aliquot of  $a_1$  is taken from the non-protein fraction, brought to the appropriate pH by the addition of alkali and further digested by the enzyme.\* The increase in amino-nitrogen is determined at regular intervals. Let the increase in amino-nitrogen be represented by  $d_0, d_1, d_2, \dots d_{n-1}$  at the end of the 1st, 2nd and 3rd and  $n$ th hour.

Then at the end of

1st hour of hydrolysis,  $b_1 - b_0 = X_1(a_1 - a_0)$

2nd     ,,     ,,      $b_2 - b_1 = X_2(a_2 - a_1) + d_1$

$n$ th hour     ,,      $b_n - b_{n-1} = X_n(a_n - a_{n-1}) + d_{n-1}$

where  $d_{n-1}$  is the increase in amino-nitrogen of  $a_{n-1}$  from  $n - 1$ th to  $n$ th hour.

In the equation  $b_n - b_{n-1} = X(a_n - a_{n-1}) + Y(a_{n-1})$

$Y(a_{n-1}) = d_{n-1}$ . Hence the true amino-nitrogen of ( $a_n - a_{n-1}$ ) which is equal to  $(b_n - b_{n-1}) - d_{n-1}$  can be found out.

$$\therefore C_6 = \frac{(a_n - a_{n-1})}{(b_n - b_{n-1}) - d_{n-1}}.$$

By the above procedure, it is possible to calculate the correct complexity factor  $C_6$  for  $a_n - a_{n-1}$ ; and hence one can get an idea of the nature of protein degradation products not precipitated by trichloroacetic acid during a particular interval of time between the  $n$ th and  $(n - 1)$ th hours.

#### Applications of the Procedure.

The procedure was applied to the experimental results of different workers to determine the nature of the degradation products of casein. The values were taken from the papers of Northrup (1933) and Bhagvat (1936).

\* It is necessary to study the influence of the sodium salt of the trichloroacetic acid, in the concentration present, on the hydrolysis.

*Bhagvat's Results.*

Substrate concentration—1.4 per cent. ; Enzyme—Crude trypson.  
Temperature 30° C. pH 7.7.

Time in mins.	C <sub>4</sub>	C <sub>5</sub>
0	..	..
10	12.1	..
20	10.2	9.3
40	9.1	7.4
60	8.8	6.8
120	7.9	5.2
240	7.3	3.9

*Northrup's Results.\**

Enzyme—Crude trypsin. Temperature 35° C. pH 7.6.

Time in mins.	Substrate concentration (percentage)					
	1.25		2.5		5.0	
	C <sub>4</sub>	C <sub>5</sub>	C <sub>4</sub>	C <sub>5</sub>	C <sub>4</sub>	C <sub>5</sub>
15	15.1	..	14.2	..	14.9	..
30	11.7	7.6	13.9	11.5	12.5	9.5
45	11.5	10.9	13.4	15.9	13.3	16.0
60	10.3	4.1	12.4	9.4	13.4	12.7
75	10.0	8.4 ?	12.2	8.9	13.2	12.9
90	9.2	4.3	11.4	5.5	13.3	14.3
105	8.5	3.0	10.4	2.6	13.3	13.2
120	8.2	2.7	9.5	2.6	12.6	7.9
Hydrolysis	Almost complete		Almost complete		Incomplete	

\* The figures given in the table are approximate. They have been computed from the graphs given in the paper.

Enzyme—Crystalline Trypsin. Temp. 35° C. pH 7.6.

Time in mins.	Substrate concentration (percentage)					
	1.25		2.5		5.0	
	C <sub>4</sub>	C <sub>5</sub>	C <sub>4</sub>	C <sub>5</sub>	C <sub>4</sub>	C <sub>5</sub>
15	13.4	..	19.0	..	17.4	..
30	15.0	28.6	16.9	9.0	15.8	12.6
45	15.9	26.3	14.0	7.5	14.5	7.6
60	14.3	8.5	14.4	8.3	13.9	7.0
75	14.9	..	14.1	..	13.5	7.8
90	..	..	14.0	..	13.1	..
Hydrolysis	Complete		Complete		Incomplete	

The above results show that in the case of digestion with crystalline trypsin, the hydrolysis stops when C<sub>4</sub> reaches a value in the neighbourhood of 14, under the three different substrate concentrations 1.25–5.0 per cent. It stops with the polypeptide stage where peptides containing about 10 amino-acids are released. With crude trypsin on the other hand, the complexity factor is 8. Here both the results of Northrup and Bhagvat are in agreement, and this factor increases with substrate concentration. Crude trypsin is probably associated with a peptidase thus rendering a further degradation of casein particle possible than is the case with trypsin alone. Taking the factor C<sub>5</sub>, it will be observed that when the substrate concentration is 1.25 per cent., in the case of crude trypsin where the action has gone to completion, the final value is 2.6. On the other hand, with crystalline trypsin the value is about 8 and this value does not vary much throughout the period of hydrolysis. This shows that peptides with a complexity factor 8 are chipped off from casein when acted upon by crystalline trypsin.

#### Summary.

A procedure for determining the nature of the products of proteolysis making use of the factor, total N/amino N, at various stages of the enzyme action, is described.

The procedure has been applied to the results of casein hydrolysis. It has been shown that under the action of crystalline trypsin, peptides with a complexity factor of 8, are chipped off from the casein. With crude trypsin, on the other hand, no such regularity is observed.

Our thanks are due to Mr. M. Sreenivasaya for many helpful suggestions.

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# THE DEVELOPMENT OF ANURAN KIDNEY.

## Part I. The Development of the Mesonephros of *Rhacophorus maculatus*\* Boulenger.

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### Introduction.

" INVESTIGATIONS are greatly needed on the embryology of *Anura* outside the genus *Rana*.....it will be only after greatly extended studies on different species that we shall have a really comprehensive idea of typical Anuran development. "

\* The genus *Rhacophorus* includes forty-two known species. Twenty of them are found in India and its dependencies and six occur in other parts of the oriental region. Four are known from China and Japan while the rest are confined to Madagascar. The genus is so closely allied to *Rana* that their characters run into each other and Gadow considers the distinguishing characters between the two genera as unimportant. *Rhacophorus maculatus* Boulenger is confined to India and Ceylon. It is popularly known as the " Chunam Frog ". It is arboreal in habit and like *Rh. schlegelii* of Japan lays its eggs in a foamy mass on the margin of tanks.

† The terminology of Peter Gray (1930) has been employed in this work.

It is nearly 20 years since this almost complaining remark of Graham Kerr (1919) was made and yet no attempt has been made to remedy this defect. In the present paper the author proposes to deal with the development of the mesonephros in *Rhacophorus maculatus*.

Spengel (1876), Nussbaum (1880), Hoffmann (1886), Marshall and Bles (1890) and Farrington (1892) have worked on the development of the amphibian kidney. But the works of Hall (1904) and Filatow (quoted by Gray, 1930) alone were exclusively devoted to the development of the mesonephros in *Rana sylvatica* and *R. esculenta* respectively. Peter Gray (1930) worked on the development of the mesonephros of *R. temporaria*. The last author gave an excellent summary of previous work on the subject. The present author is not aware of any previous work on the development of the kidney of *Rhacophorus maculatus*.

#### *Material and Method.*

Egg masses of *Rhacophorus* were collected within the University area and were allowed to hatch and develop in the Laboratory. Later on the tadpoles were transferred to open-air tanks in the University gardens. The advanced and metamorphosing stages were procured from the open-air tanks while the earlier stages were selected from those developing in the Laboratory. The tadpoles used in the course of this work were graded according to the length from the tip of the snout to the end tip of the tail. The characteristics of the tadpoles of the selected stage-lengths are noted below :—

Stage I, Length 5-7 mm., with yolk sac.

„ II, „ 12-14 „ with external gills disappearing.

„ III, „ 18-21 „ with external gills completely disappeared.

Hind legs evident.

„ IV, „ 23-25 „ hind legs developed. Front legs evident.

„ V, „ 36 „ with front and hind legs.

„ VI, with mouth widening and tail disappearing.

Further the various stages in the formation of the mesonephros seem to correspond to the different stage-lengths of tadpoles selected out as above.

Bouin's fluid was invariably used for fixing the material. The material was left in the fixative for 10-12 hours. It was then washed in 70 per cent. alcohol till the yellow colour was removed. After dehydration it was cleared in cedar wood oil. The usual method of embedding in paraffin was followed. In the case of advanced tadpoles where the skull formation has begun, the material was left in 2.5 per cent. nitric acid in 70 per cent. alcohol for about 15 days. Then it was washed in 70 per cent. alcohol till there was no trace of acid. The intestines of the tadpoles at this stage always contain grit. The

entire gut was therefore completely removed before embedding. In the case of these advanced tadpoles cold impregnation with xylol and paraffin was also used. Further these were left in the bath from  $1\frac{1}{2}$  to 2 hours whereas in the case of the earlier stages  $\frac{1}{2}$  hour to  $\frac{3}{4}$  hour was quite sufficient.

In all cases 12  $\mu$  sections were cut along transverse, sagittal and frontal planes. Delafield's hæmatoxylin with eosin as counter stain gave excellent results. In a few cases iron-alum-hæmatoxylin was also employed.

#### *Development of Mesonephros.*

*Stages 1 and 2.*—In stage 1, the mesonephros is represented by an irregular retro-peritoneal tract of cells the "Blastema" which occupies the dorso-median wall of the archinephric duct. As Furbringer (1887) and Gray (1930) have pointed out this tract of cells is derived from specialised mesenchyme cells. From this tract of cells the mesonephric units arise.

Series of transverse sections of stage 2 reveal the condensation of the cells of the blastema into spherical vesicles, the nephroblast vesicles. Each nephroblast vesicle is composed of 10 to 12 loosely packed blastema cells. There are 6 to 8 vesicles on either side. This number varies in different tadpoles I have examined. In no case however, I found more than 8 vesicles. These vesicles do not have any segmental arrangement. To begin with each vesicle is spherical. Later on due to the reorientation of the inner mass of cells they assume a oval shape.

The nephroblast vesicles seem to arise at the same time. There is no indication whatever to show that the posterior units are developed earlier than the anterior ones. In fact, there is no regular line of development of these units after their appearance. In some, posterior ones are in a much advanced stage of development. There are also cases where anterior and central units show more advanced development than the posterior units.

As in the case of *Rana* (Gray, 1930) the units of the left side are invariably better developed than those of the right side. This asymmetry becomes even more prominent (or pronounced) in the development of the later units.

Each nephroblast vesicle then develops a lumen and elongates at either end. The end near the archinephric duct forces its way into it and the other end grows downwards towards the peritoneal wall. At the same time the latter end develops a dilatation as a result of the proliferation of the cells at its free end. These cells (R.M.G. Fig. 1) are the rudiment of the malpighian glomerulus. This cellular mass grows inwards into the lumen of the growing tubule and ultimately severs its connection with the tip (E.M.C. Fig. 2). Thus a completed glomerulus results. But it will be noticed that the glomerulus results a vascular connection throughout its functional condition.

Fig. 1 shows the formation of the malpighian glomerulus. Outside the wall of the malpighian capsule is another thickening (R.F.N.) These are proliferated from the squamous epithelium of the malpighian capsule. This is the rudiment of the early peritoneal funnel.

Now as the cells of the walls of the tubule divide the tubule increases in length and is thrown into a characteristic 'S'-shaped loop. This is the 'Henle's loop' of other forms. The later coiling of the tubule becomes too complicated to follow. In Figs. 1, 2 and 3 transverse sections of these coiled tubules (T) are seen.

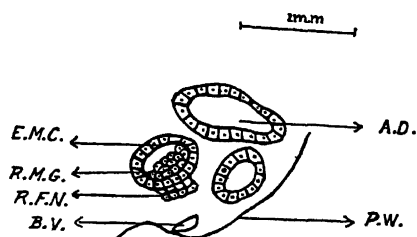


FIG. 1.—The development of the funnel rudiment in connection with the squamous epithelium of the malpighian capsule.

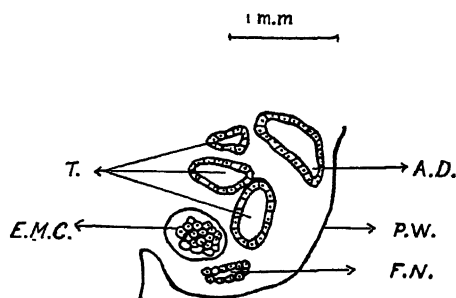


FIG. 2.—The funnel rudiment lying separated from the squamous epithelium.

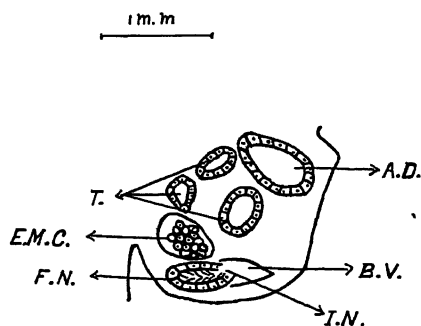


FIG. 3.—The early peritoneal funnel with its inner opening into the blood-vessel.

Stage 3.—Towards this stage as a result of growth and coiling of the nephroblast vesicle tubule the malpighian capsule with its glomerulus is pushed towards the peritoneal wall. During this process the group of cells (R.F.N. Fig. 1) which were proliferated from the squamous epithelium of the malpighian capsule get detached from the wall of the capsule and lie very close to it (F.N. Fig. 2). In this condition they appear as if they were a condensation from the blastema cells. Then there takes place a reorientation of the cells resulting in the formation of a lumen within them. The plane of the small tubule thus formed is transverse to the malpighian

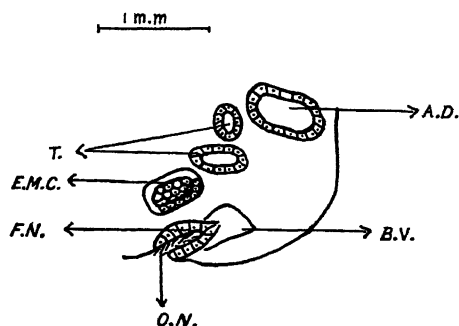
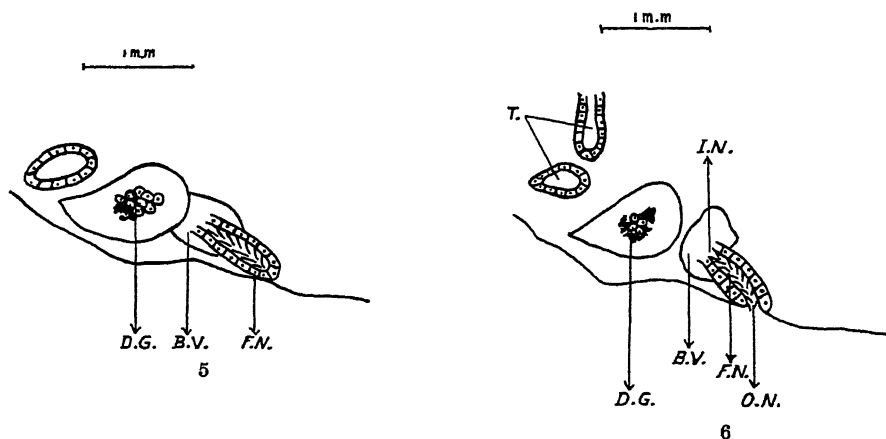
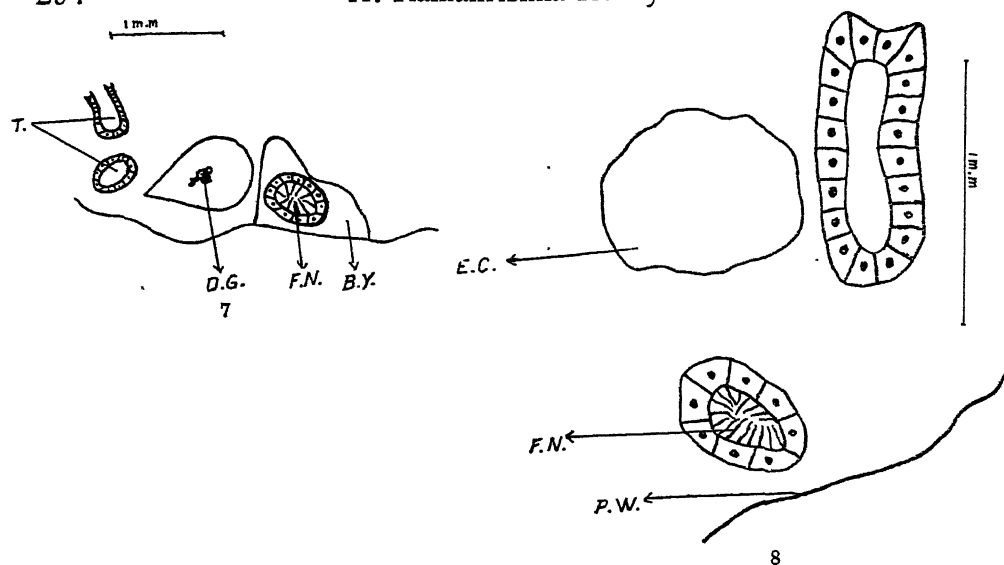


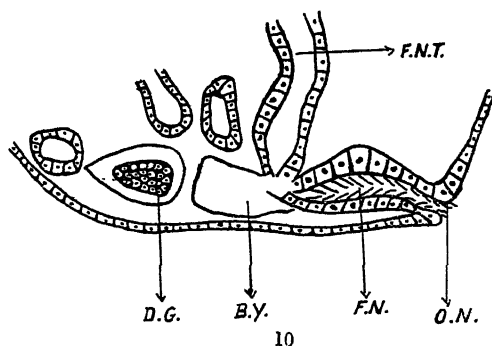
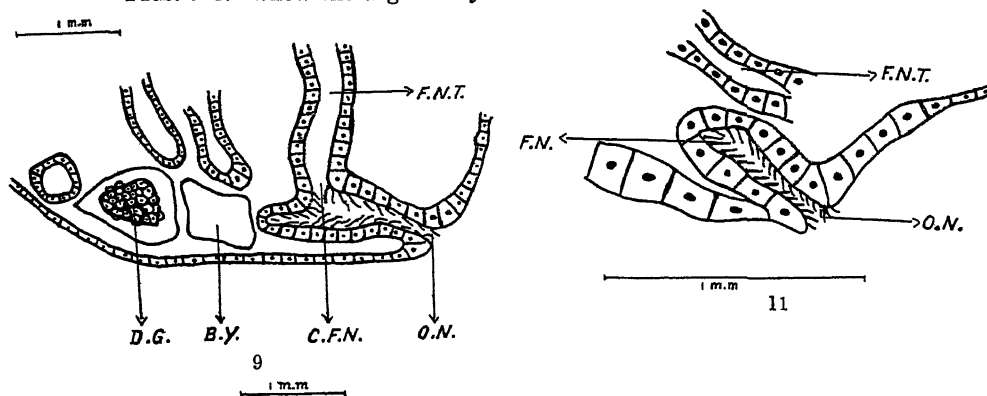
FIG. 4.—Shows the outer opening of the early peritoneal funnel.

capsule. Cilia are now developed in the lumen of the tubule (F.N. Fig. 3). As the malpighian capsule approaches the peritoneal wall this ciliated tubule or peritoneal funnel of the early malpighian units is wedged in between them. It ruptures the peritoneal wall and establishes a communication with coelom (O.N. Fig. 6). Its inner end (I.N. Figs. 3, 5 and 6) opens into a blood vessel. Here the peritoneal funnels like those of *Rana* establish a direct communication between the coelom and blood-vessels.





FIGS. 5-8.—Show the degeneration of the early malpighian glomerulus.



FIGS. 9-11.—Illustrate the mode of constriction of the later peritoneal funnel from the funnel-forming tubule.

N.B.—Read 'B.V.' in the place of 'B.Y.' in Text-Figs. 7, 9, 10, 13, 15 and 20.

As these early mesonephric units are developed the archinephric duct is pushed away from the blastema. But there are 4 to 5 outgrowths from the archinephric duct which maintain a connection between the two. Each one of these outgrowths to start with arises from condensations of blastema along the dorso-median side of the archinephric duct resembling those of the rudiments of the early nephroblast vesicles (F.St. Fig. 16).

Stage 4.—The condensations (F.St. Fig. 16) on the archinephric duct develop into well-defined straight tubules (St. Figs. 12, 18 and 19). Their lumina become continuous with the lumen of the archinephric duct (A.D. Figs. 19 and 12). At the growing end of the straight tubule is a group of cells (A.M.C. Figs. 12 and 19) which resemble the condensation of blastema cells from which early malpighian capsules are developed. But this rudiment never gets perfected. This is the abortive malpighian capsule described by Gray (1930) in the case of *Rana*.

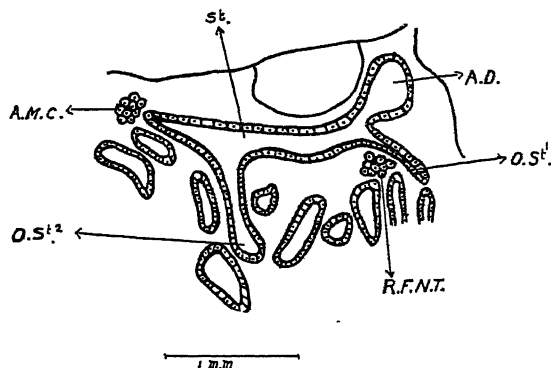
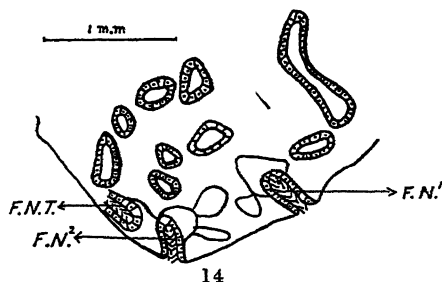
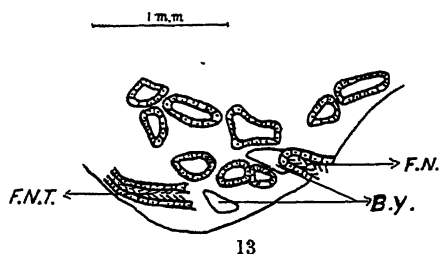


FIG. 12.—The straight tubule with two outgrowths.

Along the length of each straight tubule outgrowths or secondary tubules (O.St¹, O.St². Fig. 12) are developed at regular intervals. In most cases only three outgrowths are developed. The lumen of each secondary tubule is continuous with that of the straight tubule. At the free end of the straight tubule is the abortive malpighian capsule (A.M.C.).



FIGS. 13 and 14.—Show the formation of lateral peritoneal funnels from the funnel-forming tubule.

In this stage the blastema, which has been separated from the archinephric duct, is arranged in the form of dorsoventrally hanging tracts from the straight tubules. In each tract generally three and rarely four condensations of the blastema cells—the capsuleblast vesicles—appear. The first vesicle which is formed is pushed downwards by the other two which are formed above it a little later. The condensations give rise to the later malpighian capsules.

In Fig. 15 is a string of three malpighian capsules ( $M.C.^1$ ,  $M.C.^2$  and  $M.C.^3$ ). The lowest capsule is the best developed. This is the first formed capsule which has been pushed down by the formation of the other two capsules above it. Just in front of the lowest malpighian capsule ( $M.C.^3$ ) is a peritoneal funnel ( $F.N.$ ). In connection with the middle malpighian capsule ( $M.C.^2$ ) is another funnel which is cut in a transverse plane. In connection with the uppermost malpighian capsule ( $M.C.^1$ ) is a condensation of cells ( $R.F.N.$ ). This is the rudiment of the peritoneal funnel to be developed later on.

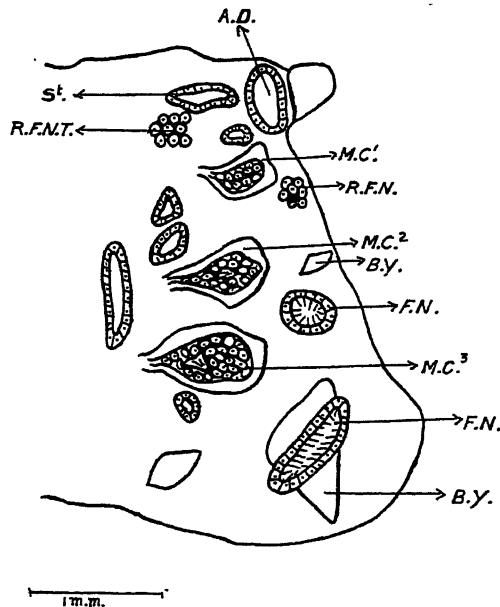


Fig. 15.—Shows a string of later malpighian capsules with their corresponding peritoneal funnels developed between them and the peritoneal wall.

The structures outlined above are formed in the way:—The lowest capsuloblast vesicle develops into malpighian capsule. As the other two capsuloblast vesicles above it are developed it is pushed downwards towards

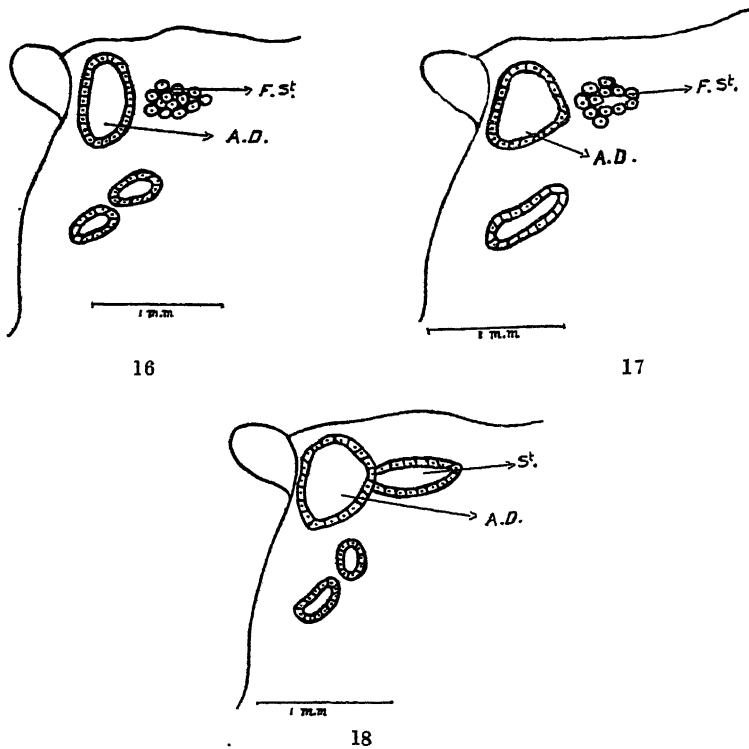
the peritoneal wall. As each malpighian capsule approaches the peritoneal wall a condensation of the blastema cells is distinguished between the malpighian capsule and the peritoneal wall. This condensation gives rise to the peritoneal funnel which is found in connection with every later malpighian capsule.

This condensation or the rudiment of the later peritoneal funnel is formed near the peritoneal wall itself. It is not formed in front of the malpighian capsule and carried to the peritoneal wall as the capsule is pushed downwards as in the case of *Rana* (Gray, 1930). When the malpighian capsule is away from the peritoneal wall no condensation can be made out in front of the capsule. Neither is there one near the peritoneal wall. But as the malpighian capsule approaches the peritoneal wall the condensation makes its appearance near the peritoneal wall. The approach of the capsule appears to stimulate or initiate the condensation of the blastema cells near the peritoneal wall. Further development of the rudiment of the peritoneal funnel is similar to the process outlined by Gray (1930) for *Rana*.

Each malpighian capsule develops a small tubule at the end opposite to that which faces the peritoneal wall. This tubule grows upwards and fuses with an outgrowth from the straight tubule. Thus each malpighian capsule indirectly communicates with the lumen of the archinephric duct through the straight tubules and then outgrowths just as described for *Rana* by Gray (1930). As has been pointed out by Gray (1930), the straight tubule with its outgrowths is the collecting trunk of earlier authors.

Examination of a series of transverse and longitudinal sections of this stage reveals that the early malpighian capsules which maintained a direct communication have by now disappeared. In somewhat earlier sections can be made out degenerating tubules attached to the dorsomedian wall of the archinephric duct. Towards the peritoneal wall are also seen malpighian capsules which have lost their blood connection, in a state of degeneracy. Figs. 5, 6, 7 and 8 illustrate this process of degeneration and disappearance of the early malpighian capsules (D.G.). Their function is now taken up by the strings of later malpighian units. But the peritoneal funnels of the early units do not degenerate. They alone persist of the entire early mesonephric units (F.N. Figs. 5, 6, 7 and 8).

*Stages 5 and 6.*—Serial sections of these stages show that the peritoneal funnels of the later mesonephric units outnumber the malpighian capsules. The occurrence of this large number of peritoneal funnels is brought about in the following manner :—



FIGS. 16-18.—Show the origin of the rudiment of the straight tubule and its subsequent development into the straight tubule.

Lying in the course of the blood-vessel are the coils of a tubule. This tubule to begin with arises from a group of cells (R.F.N.T. Fig. 12). This is the rudiment of the funnel-forming tubule. This tubule does not form any connection at all with the straight tubule. The tubule grows ventrally, closely following the peripheral blood-vessels. The lumen of its lower extremity is ciliated and lies parallel to the peritoneal wall. On reaching the peritoneal wall the ciliated tip is constricted off (F.N. Figs. 9, 10 and 11). The outer end of the severed tip establishes a connection with the coelom (O.N. Figs. 9, 10 and 11). The inner end opens into the blood-vessel (I.N.). Thus the tubule lying in the blood-vessel is the funnel forming the tubule described by Gray (1930) in *Rana*.

In Fig. 13 is the first funnel (F.N. Fig. 13 and F.N.<sup>1</sup> Fig. 14) which has been developed from the funnel-forming tubule (F.N.T.). Fig. 14 shows two peritoneal funnels (F.N.<sup>1</sup> and F.N.<sup>2</sup>) which have been developed from the funnel-forming tubule (F.N.T.). Figs. 9, 10 and 11 show the actual process by which a peritoneal funnel is constricted off from the funnel-forming tubule.

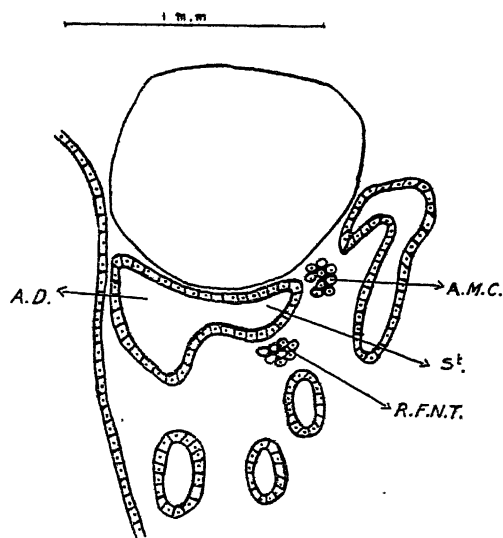


FIG. 19.—The straight tubule which has acquired its connection with the archinephric duct.

After giving rise to the peritoneal funnel (F.N. Fig. 11) the tip of the tubule (F.N.T. Fig. 11) turns upwards. At some distance from the first peritoneal funnel (F.N.<sup>1</sup> Fig. 14) it gives rise to another funnel (F.N.<sup>2</sup> Fig. 14).

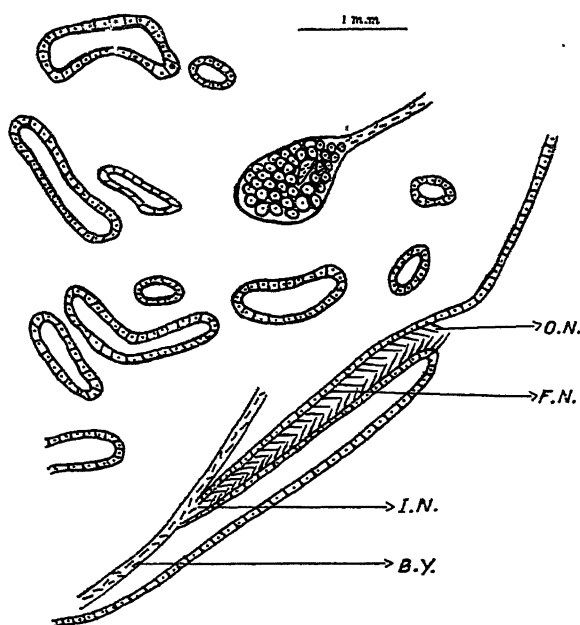


FIG. 20.—An abnormal peritoneal funnel showing a very long tail,

The formation of the peritoneal funnels in this manner is not indefinite. Each tubule as far as I have examined never gave rise to more than three peritoneal funnels at this stage.

Thus in a metamorphosed *Rhacophorus* there are three kinds of peritoneal funnels :—

(1) The peritoneal funnels of the early mesonephric units, which persist. These are derived from the proliferation of the cells of the squamous epithelium of the early malpighian capsules.

(2) The peritoneal funnels which are developed in connection with the later malpighian capsules. These are derived from groups of blastema cells which are condensed near the peritoneal wall under the influence of the approach of the malpighian capsule towards the peritoneal wall.

(3) The peritoneal funnels which are developed from the funnel-forming tubule.

#### *Discussion.*

The development of the mesonephros in *Rhacophorus* is almost similar to that of *Rana* (Gray, 1930). To begin with, we have the formation of the early malpighian units from nephroblast vesicles which have a direct communication with the archinephric duct. These units later disappear and we then have the development of the later malpighian units. Here the communication of the units with the archinephric duct is indirect. The malpighian capsules are connected with the archinephric duct by means of the straight tubules and their outgrowths.

*Formation of the Peritoneal Funnels.*—A consideration of the formation of peritoneal funnels shows certain deviations from those of *Rana*. The rudiments of the peritoneal funnels of the early units are developed from the thickenings of the squamous epithelium of the external walls of the malpighian capsules. The cells which are proliferated become columnar and get detached from the capsular wall. The rudiment then elongates and develops a lumen. It is pushed by the downwardly growing malpighian capsule towards the peritoneal wall, where it lies parallel to the latter. The lumen gets ciliated and opens into the coelom externally and into the blood-vessel internally.

The origin of the funnel-forming rudiment is different from that of *Rana*. It arises as a condensation of blastema cells in front of the malpighian capsule and is later carried to the peritoneal wall. There is no difference in the further development of the funnel.

The formation of the rudiments of the early peritoneal funnels is comparable to that of the urodelan peritoneal funnels (*Triton*, Gray, 1933). But in

*Triton* the rudiment remains in connection with the malpighian capsule and establishes a connection between the coelom and the cavity of the malpighian capsule.

The main part of the development of the peritoneal funnels in connection with the later malpighian capsules is similar to that of *Rana*. But here in *Rhacophorus* the blastema cells are not condensed in front of the malpighian capsule and carried to the peritoneal wall. The condensation of the blastema cells takes place near the peritoneal wall itself as the malpighian capsule approaches the peritoneal wall.

The production of the peritoneal funnels by the funnel-forming tubule is exactly similar to that described for *Rana*. In all the three types the peritoneal funnels never communicate with the cavity of the capsule but invariably establish a connection with the coelom on the one hand and the blood-vessel on the other.

*Function of the Peritoneal Funnels.*—Gray (1932) has explained the establishment of a direct communication between the coelom and the blood circulation by the peritoneal funnels, by attributing a very important function of collecting a secretion from the coelom and passing it on to the blood circulation. In fact, Gray (1932) considers this function as a primary one and the process of excretion by the peritoneal funnels as only of secondary importance. The present author is in complete agreement with this view.

This direct communication between the blood circulation and the coelom cannot be without some physiological significance. It is impossible to see how such a connection is helpful, in a process of excretion. This certainly points to an important function other than that of excretion by the peritoneal funnels. Then we are also faced with their prodigious multiplication. If they were merely excretory the funnels developed in connection with the malpighian capsules would have been quite sufficient. The multiplication of the funnels indicates the primary nature of this collection and conduction of the secretion from the coelom.

In *Rhacophorus* we have the retention of the peritoneal funnels of the early units, when the entire early mesonephric units have disappeared. Gray (1930) however has not stated whether the early peritoneal funnels are retained or not in *Rana*. When there is need for more peritoneal funnels and when there is the development of a special tubule towards their multiplication, why should already functioning early peritoneal funnels disappear?

In *Rhacophorus* it is noted that its larval life after the fore legs have become evident, is very short. In fact, their appearance heralds the end of

its larval life. Immediately afterwards the mouth widens, tail disappears and the metamorphosis is completed. It will be remembered that it is at this stage that the funnel-forming tubule is developed and the peritoneal funnels are multiplied. This results in bringing about a rapid collection and conduction of the important secretion from the cœlom.

Can it be possible then, that this secretion is in some degree responsible in shortening the larval life of the animal? If it were possible we have to expect the disappearance of at least some of the funnels after the metamorphosis is completed and the adult stage is reached. But there is no indication of such a disappearance in the metamorphosed animal. In the adult condition it has been demonstrated in other forms that these funnels are found in a very active state and Gray (1936) has pointed out recently the development of even accessory peritoneal funnels in the post-metamorphic kidney of *Rana* in addition to the already existing peritoneal funnels. Hirt (1930) has also shown the existence of an extensive nerve-net correlated with the presence of these funnels in the adult animal.

The development of the mesonephros of *Rhacophorus* and other Anura proceeds along such lines as to bring about a rapid collection and conduction of this important secretion from the cœlom into the blood circulation. In what manner this secretion is important we are at present neither in a position to state nor is it our concern in this communication. But this much can be said. This secretion is essential to the animal both in its larval and adult life. Even in Urodela this secretion is collected from the cœlom by the peritoneal funnels and conducted in an indirect manner into the blood circulation. The long larval life of Urodela does not necessitate the multiplication of the peritoneal funnels such as seen in Anura for the collection of this secretion.

In the face of a short larval life Anura have evolved a process of multiplication of these funnels to effect a rapid collection of this secretion from the cœlom. Further the efficiency of the conduction of this secretion into the blood circulation is enhanced by the establishment of a direct communication between the blood circulation and the cœlom.

There is no doubt whatever that the Anuran mesonephros is evolved from a Urodelan type. The modification of the mesonephros in Anura is conditioned by different life-history of the animals. The formation of the rudiments of the peritoneal funnels of the early mesonephric units of *Rhacophorus* are certainly to be regarded as pointing towards Urodelan ancestry.

#### Summary.

(1) The general development of the mesonephros in *Rhacophorus* is similar to that of *Rana* (Gray, 1930).

- (2) The early malpighian units arise from nephroblast vesicles.
- (3) Each nephroblast vesicle is a condensation of 10–12 loosely packed blastema cells.
- (4) Six to eight nephroblast vesicles are developed.
- (5) Each early peritoneal funnel arises as a thickening of the squamous epithelium of the early malpighian capsule.
- (6) This thickening later severs its connection with the malpighian capsule, develops a lumen and opens externally into the cœlom and internally into a blood-vessel.
- (7) The early peritoneal funnels persist.
- (8) The early malpighian capsules and their tubules degenerate and disappear.
- (9) The later malpighian capsules arise from capsuloblast vesicles.
- (10) A string of three capsuloblast vesicles appears in a dorso-ventrally extending tract of blastema near each straight tubule.
- (11) The straight tubules and their outgrowths arise from 4 to 5 condensations of blastema on the dorso-median wall of the archinephric duct.
- (12) At the free end of the straight tubule is an abortive malpighian capsule which never reaches perfection.
- (13) The peritoneal funnels in connection with later malpighian capsules are developed from condensations of blastema cells.
- (14) The condensation of the blastema cells does not take place in front of the malpighian capsule as has been described in *Rana*; but takes place near the peritoneal wall as the malpighian capsule approaches the peritoneal wall.
- (15) The later peritoneal funnels also establish a direct communication between the cœlom and the blood circulation.
- (16) A funnel-forming tubule arises from a condensation of cells near the straight tubule.
- (17) Thus tubule never gets connected with the archinephric duct.
- (18) Its lower tip which lies parallel to the peritoneal wall gets ciliated and follows the course of the blood-vessels closely.
- (19) By a repeated process of constriction it gives rise to three peritoneal funnels which also open into the blood-vessels internally and into the cœlom externally.
- (20) The present author agrees with the view put forward by Gray (1932) that the peritoneal funnels have a primary function of collecting an important secretion from the cœlom and conducting it into the blood circulation.

(21) It is suggested that in *Rana* as in *Rhacophorus* the early peritoneal funnels might persist.

(22) The formation of the rudiments of the early peritoneal funnels of *Rhacophorus* are regarded as pointing towards Urodelan ancestry of the mesonephros.

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REFERENCE LETTERS.

A.D.	Archinephric duct.
A.M.C.	Abortive malpighian capsule.
B.	Blastema.
B.V.	Blood vessel.
C.F.N.	Point at which the peritoneal funnel is constricted off.
D.G.	Degenerating malpighian glomerulus.
E.C.	Capsule of the early malpighian unit in which the glomerulus has completely disappeared.
E.M.C.	Early malpighian capsule.
F.N.	Peritoneal funnel.
F.N. <sup>1</sup> & F.N. <sup>2</sup>	Peritoneal funnels produced from funnel-forming tubule.
F.N.T.	Funnel-forming tubule.
G.	Glomerulus.
I.N.	Opening of the peritoneal funnel into the blood-vessel.
M.C. <sup>1</sup> , M.C. <sup>2</sup> & M.C. <sup>3</sup>	Later malpighian capsules developed from capsuloblast vesicles.
O.N.	Opening of the peritoneal funnel into the coelom.
O.St. <sup>1</sup> & O.St. <sup>2</sup>	Outgrowths of the straight tubule.
P.W.	Peritoneal wall.
R.F.N.	Rudiment of the peritoneal funnel.
R.F.N.T.	Rudiment of the funnel-forming tubule.
R.M.G.	Rudiment of the early malpighian glomerulus.
R.St.	Rudiment of the straight tubule.
St.	Straight tubule.
T.	Tubules.

# FELSPARS FROM THE PEGMATITES OF KODARMA, BIHAR.\*

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## I. Introduction.

THE specimens of feldspars which form the subject of this paper, were collected by the geology students of the Indian School of Mines during the field-seasons of 1932, 1933 and 1935, while preparing the geological map of the Government Reserve Forest, Kodarma, under the guidance of Prof. S. K. Roy. A short description of these feldspars was given by Messrs. H. G. Pathak and Hamzah Bhai in their A.I.S.M. theses on the Economic Minerals of Kodarma Pegmatites. The collection of feldspars was re-examined by the author in 1937 at the University of Liverpool under the kind guidance of Prof. H. H. Read. The present paper describes and discusses the origin of the various perthitic intergrowths observed in these feldspars.

## II. Classification of the Feldspars.

The common feldspars of the pegmatites of Kodarma are pink and white microcline, and white or greyish-white albite-oligoclase. Green microcline, as the main constituent of a pegmatite, is only occasionally met with.

Most of the specimens when examined under the microscope, show perthitic intergrowths of soda and potash feldspars. So far as the writer is aware, Anderson<sup>1</sup> is the only mineralogist who has given a detailed description of the textural features of micro-perthites, and therefore his nomenclature of the perthites has been followed here in classifying the Kodarma feldspars.

Based on their textural characters, the feldspars of this area may be classified into the following four groups :—

- (i) Orthoclase perthite of the composite 'vein' and 'film' type.
- (ii) Microcline perthite of the composite 'vein' and 'film' type.
- (iii) Albite-oligoclase 'antiperthite'.
- (iv) 'pure' Albite-oligoclase.

This is, however, no clear-cut classification. The first type of feldspar passes into the second with the complete inversion of orthoclase into microcline. Similarly, the third type passes into the fourth one when there is

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\* Summary of a part of the M.Sc. thesis, accepted by the University of Liverpool (1938).

complete absence of microcline in it. The first two types, however, are dominantly rich in potash, whereas the last two are rich in soda.

There is yet another difference, as observed in the field, between the first two and the last two types of felspars. Workable deposits of 'ruby' mica occur usually in those pegmatites which contain felspars of type (iii) or (iv), and only rarely in those containing felspar of type (ii). No deposit of mica is known to occur with felspar of type (i). This association has an important bearing on the origin and prospecting of the mica-pegmatites.

### III. Description of the Felspars.

(1) *Orthoclase perthite*.—Orthoclase is one of the rare felspars in the Kodarma pegmatites, and most of its specimens are observed under microscope to have undergone inversion into microcline. Thin veinlets or stringers of soda-felspar (albite) are easily distinguished under the microscope by their having slightly higher relief and birefringence than the orthoclase host. In 001 sections of the felspar, these veinlets are rather curved and run serpent-like in the orthoclase (Pl. XII, Fig. 1). The bigger veinlets show albite type of twinning under high magnification, and they are generally elongated perpendicular to their twinning direction. Incipient microclitic twinning has sometimes developed in orthoclase at some places, but only near the edge of these veinlets.

In 010 section of felspar, the veinlets occur as much thinner, more regular, almost straight and parallel, long spindles making an angle of  $65^{\circ}$  to  $68^{\circ}$  with 001 cleavage of orthoclase. The spindles are not more than .05 mm. in thickness but their length may be as much as 2 mm. These spindles traverse the section in a series, one after the other, the second one being a bit off set from the adjoining one with a little overlap. In other words, they have an 'en echelon' arrangement (Pl. XII, Fig. 2). In the space between the two portions of the off-set spindles, there are often minute thread-like stringers, about 0.1 mm. long and .02 mm. thick.

Between the thin spindles and stringers of albite, there are still thinner lines—'films'—of soda-felspar which can be observed only under high magnification. They are evenly distributed in the orthoclase, and make an angle of  $70^{\circ}$  to  $73^{\circ}$  with its 001 cleavage. In some specimens of felspars, thin microscopic spindles of quartz and needles of muscovite are seen to have arranged themselves more or less along the 001 cleavage planes of orthoclase. The quartz spindles are in optical continuity with one another, but they cut the veinlets, and hence, are of later generation.

Further, there are a few small patches of albite scattered in the orthoclase. The veinlets of albite usually meet these patches. They, however,

do not actually cross them. The composition of these patches is almost the same as that of the veinlets, but they have slightly different optical orientation from the veinlets, as is shown by their extinction being not quite simultaneous with that of the veinlets. These patches probably represent the beginning of the formation of the normal veins of albite, observed in microcline perthites. Those veins are bigger in size than the veinlets in orthoclase perthite, and they show an irregular and replacing margin against the host. Most of these patches of albite have been the chief centres of replacement, and they are partly replaced by magnetite, tourmaline, muscovite, biotite or quartz.

An estimate of the proportions of the soda and potash feldspars in this type of perthite by Leitz' Integrating stage, gave 21-22 per cent. of albite (excluding that present as 'films') and 78-79 per cent. of orthoclase.

(2) *Microcline perthite*.—Thin sub-parallel veins of the soda-feldspar can be usually seen even in hand-specimens of these feldspars. This is due to the higher refractive index and more white colour of the veins than those of the microcline. The veins are very easily distinguished on a polished surface of the feldspar.

In this type of feldspar, the thin veins of albite (sometimes albite-oligoclase) which traverse the microcline, are neither so regular and sharp margined, nor are they so evenly distributed as those in the orthoclase perthite, described above. They are also fewer in number, are more widely spaced and are larger in size than the veinlets in orthoclase. But all the veins are in optical continuity with one another. In some specimens, microcline is kaolinized but the albite veins are fresh, whereas in others, the reverse is the case. Microcline usually shows more kaolinization in 010 and 100 sections than in 001 section, and the veins of albite are usually more kaolinized along their margin than in the centre, though at places where the albite veins have swelled out, their core also may be much kaolinized. In some cases, portion of microcline just near the albite-veins is comparatively less kaolinized than that far from the veins.

In 001 sections, microcline usually shows typical cross-hatch twinning, but sometimes broad untwinned lamellæ (lying between two pericline twin-planes) alternate with cross-twinned ones. Veins of albite mostly occur in the twinned portions of microcline (Pl. XII, Fig. 3). Occasionally near the margin of these veins, the broad untwinned bands of microcline are traversed by a couple of large spindle-shaped albite-twin lamellæ projecting from the sides. It is, therefore, suggested that amongst the two sets of twinning, the albite-twinning is probably a bit later than the pericline one,

and that the albite veins may be partly responsible for the development of cross-twinning in microcline.

The margin of the albite-veins with microcline is very sinuous. These veins often send out tongues or protrusions into the microcline, two sides of which are parallel to the two twin-planes of the latter mineral. There are sometimes many irregular or rounded 'islands' of microcline enclosed in some of the veins. These islands are all in optical continuity with one another and with the main microcline. The albite-twinning of the veins is always parallel to that of the microcline host, but the actual twin-planes of the two felspars are not identically the same. The albite veins may be as much as 8 mm. long, but the same vein may show a thickness varying from .02 mm. to 0.6 mm. or even more. The general direction of elongation of the veins is usually perpendicular to 010, but in some cases, the veins may run at an angle varying from  $48^{\circ}$  to  $70^{\circ}$  with this direction. In unoriented sections, in which microcline shows rhombic cross-hatching, the veins may appear as broad irregular patches with no definite direction of elongation. The veins are sometimes zoned along the margin and show a sort of wavy extinction.

Microcline shows 'film' type of perthitic lines between the veins. The films lie perpendicular to 010, and are best seen in 010 sections of microcline as a set of thin microscopic lines, lying at an angle of  $70^{\circ}$  to  $74^{\circ}$  with the 001 cleavage lines of microcline. They are usually .01 mm. to .02 mm. in thickness and about 0.1 to 0.3 mm. in length (Pl. XIII, Fig. 1). It has been observed that the microcline nearest to the albite veins is almost free from films of albite, which are abundant in the middle portion between two veins. This indicates that the albite of the films probably diffused to the wider veins during the formation of the latter.

In many sections of pink microcline perthites, 'inclusions' of albite-oligoclase with allotriomorphic or hypidiomorphic outline are observed. They show both carlsbad and albite types of twinning. They are at random orientation in the microcline, and lie near or cut across the albite-veins, with which they are not in optical continuity. They sometimes occur in or near the cracks, along which later solutions have played an important part. Quartz and muscovite inclusions are often found near such inclusions of felspar. The study of the margin of all these inclusions with one another and with the enclosing microcline, suggests that they have originated from later pegmatitic solutions by the process of replacement (Pl. XIII, Figs. 2 & 3). Minute spindles of quartz and needles of muscovite lying at right angles to the films and making a small angle with 001 cleavage of microcline have been observed in this type of felspar also,

An estimate of the proportions of potash-felspar and soda-felspar (excluding that occurring in the films) was made by Leitz' Integrating stage in a few specimens of microcline perthites and the following results were obtained :—

Pink microcline-perthite—	{ Albite 20–22 per cent. Microcline 78–80 per cent.
Green microcline-perthite—	{ Albite 20 per cent. Microcline 80 per cent.
White microcline-perthite—	{ Albite 15–16 per cent. Microcline 84–85 per cent.

(3) *Albite-oligoclase antiperthite*.—Some of the specimens have well-defined platy habit. Sometimes fine twinning-striations are clearly visible on their surface with naked eye. Under microscope, this type of felspar is composed of hypidiomorphic plagioclase (albite-oligoclase) crystals, showing irregular but smooth contacts with one another. Some of the crystals show fine regular albite-twin lamellæ, some show them far apart, whereas others are either untwinned or show only a few discontinuous twin-lines here and there. This sometimes gives rise to 'chess-board'-like structure (Pl. XIV, Fig. 3). The different sections of the felspar in a slide are more or less equidimensional, but some of them may be elongated perpendicular to 010 twin lines. In this type of felspar too, sections parallel to 010 show more kaolinization than those parallel to 001.

Numerous small scattered patches of microcline are observed as inclusions in the albite-oligoclase. They look like 'islands' of microcline in the albite-oligoclase (Pl. XIV, Fig. 2). Some of these inclusions of microcline are more or less square, some have only two opposite sides regular and running in the direction of 010 cleavage lines, whereas others possess highly irregular outline. The inclusions vary in size from  $0.2 \times 0.1$  mm. to  $1.5$  mm.  $\times$   $1.0$  mm. Sometimes though the microcline patches occur in the midst of highly kaolinized albite-oligoclase, they are themselves less altered than the enclosing plagioclase. The fresh inclusions of microcline often show films of soda-felspar under high magnification.

The proportion of potash felspar to soda-felspar in the antiperthites, as determined by Leitz' Integrating stage, is 4 to 7 per cent. of microcline and 93 to 96 per cent. of albite-oligoclase.

(4) '*pure*' *Albite-oligoclase*.—Some of these felspars look quite fresh in hand specimens, but under the microscope they are full of highly turbid patches, amongst which some fresh portions may still be seen. Many of the felspar sections look as if perforated with mica which occurs in rounded

or thin flakes. Bigger flakes of muscovite are also found as inclusions at random orientation. Some of these flakes are arranged radially or have a plumose habit, suggesting their secondary origin. In some sections, inclusions of quartz are seen definitely 'eating' the felspar by sending out its tongues along the twin-planes of the latter. Quartz is often seen working its way along the cleavage planes of mica, whereas the latter is sometimes seen to enclose island-like patches of quartz in optical continuity with the quartz at the margin. From these observations it seems that later pegmatitic and hydrothermal solutions have greatly affected these felspars and that replacement phenomenon has played an important part in the formation of some of the inclusions.

The less altered specimens of felspar show fine albite-type of twinning. Sometimes felspar shows under ordinary light, alternating more kaolinized and less kaolinized twin-lamellæ. Some of the less altered lamellæ, when examined under crossed nicols with high power objective, show a few long spindle-shaped patches which are elongated parallel to 010 and have an indistinct 'en echelon' arrangement. They are untwinned but are distinguished by their lower birefringence giving grey polarisation colour of a darker shade than that shown by the enclosing albite-oligoclase. These may represent the antiperthitic inclusions of microcline which have been resorbed by soda-felspar. Some of the microcline inclusions in the antiperthite (described above) show albite-twinning only. The 'en echelon' arrangement shown by patches in this felspar may, therefore, be explained by the fact that the soda-solutions worked into the microcline inclusions along its albite-twin planes, and thus separated its adjacent lamellæ giving a sort of parallel and overlapping appearance to them.

#### *IV. Origin of Perthitic Intergrowths.*

It is now a recognised fact that perthites have been formed in various ways. The important processes suggested are (1) simultaneous crystallisation which may be eutectic or rhythmical, (2) ex-solution or unmixing of solid solutions, and (3) replacement of one felspar by another either from within or from without the system. During recent years, ex-solution has been regarded as the most important process to account for the formation of the common perthites.

(i) *The origin of albite films.*—The films of albite observed in the first two types of felspars described in this paper, are regarded to be formed by the process of ex-solution in contraction cracks as postulated by Anderson, but the present writer differs from his view that 'films' are formed after the 'veins'. In Kodarma area, 'films' seem to have been formed before the veins for the following reasons:—

(1) The films are always of microscopic dimensions, but in some specimens they seem to grade into vein-stringers. It is natural to suppose that the cracks which will be first developed in the potash feldspar on cooling, will be of capillary size and they will be filled with albite separated by ex-solution. Afterwards they are likely to be enlarged by later solutions.

(2) The films, unlike the veins, are often parallel or sub-parallel to each other. Their direction seldom varies appreciably from that making an angle of  $73^\circ$  with 001 which, according to Anderson, is one of the directions of contraction-cracks formed in feldspars on cooling. The films, therefore, coincide more with this direction than do the veins which may show an angle varying from  $45^\circ$  to  $75^\circ$  with that direction. It is natural to expect that the cracks which now accommodate these veins were enlarged later and made to vary a great deal from the natural direction of the minute contraction-cracks which had been formed earlier and filled with the films of albite.

(3) Though it is true that albite veins do not intersect the films, it is equally true that the films are never seen to cross a vein, however small the latter might be. On the other hand, the portion of the microcline, just adjacent to the veins, is often free from the films. This suggests that the albite of the films must have diffused into the veins from their immediate vicinity when the temperature of that portion of the potash feldspar rose due to an influx of the solution forming the albite veins.

(ii) *The origin of albite stringers showing 'en echelon' arrangement.*—This peculiar type of overlapping arrangement of microscopic perthitic stringers has been explained by Shaub<sup>2</sup> to be due to the rapid cooling of the potash-feldspar which did not permit the diffusion of the albite in the stringers to the initial wide bands ('veins' of Anderson). Both the stringers and the bands of albite are regarded by him to be formed by the process of ex-solution, the bands being formed at an earlier stage when the rock was cooling slowly due to supply of heat from magmatic solutions.

This "en echelon" arrangement is often present in the ex-solution films on a sub-microscopic scale, but in the type under discussion, this arrangement is shown by regular straight-margined and discontinuous stringers of albite which in size, approach more to the veins than to the films. In these sections the films also are found to be wider than those in the other perthites, and often it is difficult to say whether a particular small stringer is to be classed as a small vein or a bigger film. The writer regards this type as an intermediate form in the thermal scale between the 'film' perthite and the 'vein' perthite and probably responsible for the inversion of orthoclase to microcline. He is inclined to agree with Anderson that the regular veins

represent the early stage in the opening of the contraction-cracks giving access to circulating solutions, which is marked by re-crystallisation with little replacement. The solutions in the early stage must have simply enlarged some of the films, and as the temperature was comparatively lower, these enlarged films or veinlets of albite could not diffuse freely and therefore arranged themselves in this peculiar fashion.

(iii) *The origin of albite 'veins'.*—Of all the perthitic occurrences, the vein-like intergrowths of albite with potash-felspar have been most differently interpreted by various authors. Shaub thinks that these veins are due to ex-solution, slow enough to allow the diffusion of albite through solid microcline to the present position (veins), as well as the diffusion of microcline from the region of the veins to that from which albite diffused.

Anderson believes that the formation of the veins is connected with the development of contraction-cracks in felspar on cooling, and they are formed by circulating solutions derived from the same magma. There has been replacement, but it is not intense. Megathlin<sup>3</sup> suggests that the tongue-like veins of albite in microcline can only be explained by the replacement of microcline by albite. A. L. Anderson<sup>4</sup> also agrees to the replacement origin of the albite-veins observed in the potash-felspar. Alling<sup>5</sup> regards the vein-perthites to be formed between deuteric and hydrothermal stages. According to him, deuteric perthites belong to a closed system, and in them the blebs of felspar are formed from the liquid introduced into the host-felspar from without its crystals, but derived from the same system. In hydrothermal perthites, the solutions are introduced from without the system and they may replace part of the host.

The writer believes that the albite-veins of Kodarma perthites were guided by contraction cracks filled by ex-solution films in the earliest history of their development, when there had been very little replacement. The cracks were subsequently widened out mostly by the process of replacement.

Amongst the criteria which Bastin<sup>6</sup> and others have suggested for distinguishing 'ex-solution' textures from those resulting from replacement, the following observations suggest that replacement has played an important rôle in the formation of the albite 'veins' in Kodarma perthites:—

(1) As a rule, the veins have not got sharp smooth boundaries characteristic of ex-solution blades and, unlike the latter, there is usually an enlargement when the veins join each other.

(2) The veins usually show some relation with the twinning planes of microcline, and with the direction of contraction-cracks as determined by

Anderson. These must have served as guiding planes for the replacing solutions.

(3) The 'veins' are not evenly distributed. The ex-solution 'films', on the other hand, are always evenly distributed in the host.

(4) According to Ross' one of the evidences of replacement is that the walls on the two sides of the veins should fail to match. In the vein type of perthites of Kodarma (excluding those vein-stringers, formed in the earliest stage) the walls of the albite veins never match on the two sides.

(5) Unlike a simple fracture-filling vein, the margins of the irregular albite-veins show protrusions into the microcline which have got their contact lines with microcline exactly parallel to one or both of the twin-planes of the latter. This suggests that the original fractures must have been widened by replacement along the twin-planes of the host.

(6) 'Islands' in parallel orientation with other 'islands' and with the adjacent 'mainland' are regarded by Bastin and others as an evidence of replacement. The wider veins of albite do contain many such islands of microcline. Some of the islands of microcline are rounded, whereas others possess irregular form with intrusions of albite-veins parallel to the twin-planes of the microcline of the islands, suggesting as if the latter were 'eaten' along the twin-planes by the invading solutions. On the other hand, rounded island-like patches of albite in microcline are never observed.

One of the facts which requires explanation is the parallel orientation of the albite of the veins with the microcline host. This is seen by the fact that twin-planes of the albite-veins are always parallel to the corresponding twin-planes of the microcline. This may be taken as an evidence for simultaneous crystallisation of the two feldspars. It is, however, noted that the actual twin-planes of one feldspar do not usually pass through the other. It is often the case that when the albite-type of twin-lamellæ of microcline on the two sides of a vein are thinner, the corresponding twin-lamellæ in the albite-vein are thicker and *vice versa*. It has been suggested in the descriptions of the orthoclase and the microcline perthites that the albite veinlets have played some part in the development of microcline twinning. It may be that when this microcline (which many American petrologists believe to be inverted orthoclase) assumed its triclinic form, its molecular arrangement must have been guided by that of the albite stringers already present, that is, the inversion of the potash-feldspar probably took place during or just after the formation of the veins. Anderson has also observed that there has been re-crystallisation of the feldspars during the formation of the veins.

The fact that there is very little variation in the proportion of albite (15 to 25%) in the vein perthites of Kodarma is against the view that the veins of albite are formed by simple replacement from outside solutions. The surprisingly small variation in the average composition of the vein-perthites from the granite pegmatites has already been pointed out by Vogt and Anderson as a result of a number of chemical analyses. The central figure of Vogt is 25% of soda-felspar, and of Anderson 23% with only 10% variation on either side. Though no chemical analysis of the Kodarma perthites was carried out, the determinations of the proportions of the two felspars in some perthites by Lietz' Integrating stage suggests that their composition would not fall outside the range of Vogt and Anderson. Anderson suggests that this relative constancy in composition of the vein-perthites may be due to the fact that the material now present in the veins may have been largely derived from the felspar itself, but complex re-crystallisations have taken place due to the formation of cracks and fluxing agents playing in them, giving rise to the peculiar textural features, which cannot be explained by ex-solution and the albite being transported to its present place in the solid state. Thus, the vein perthites of Kodarma may be regarded to belong to a closed system, but they differ from the true deuteric perthites of Alling in the greater degree of replacement of the host.

(iv) *The origin of inclusions of albite-oligoclase.*—The hypidiomorphic or allotriomorphic sections of albite-oligoclase which have been observed as inclusions in most of the vein-perthites, lie at random orientation and do not extinguish simultaneously with the albite of the veins. They always show a sinuous margin with both the microcline and the albite-veins, and commonly lie across or near the veins. They belong to a different episode in the evolution of the perthite. The patches of albite found associated with the regular veinlets in orthoclase-perthite probably represent the earliest stage of the formation of the bigger veins of albite which are seen to have enlarged themselves by the process of replacement in the case of microcline perthites. The composition of the big inclusions of plagioclase (seen in the microcline-perthites), is more towards oligoclase than that of the veins. These inclusions lie in contact with, or even cut across, the albite veins. They sometimes contain minute relic pieces of the vein-albite, which are in optical continuity with the outside veins. These inclusions of plagioclase are, therefore, definitely of later origin, and they represent crystals of albite-oligoclase formed inside the potash-felspar by replacement. They were possibly derived from the same soda-rich fraction of the pegmatitic residue from which the albite-oligoclase felspar of the mica-pegmatites originated.

(v) *The origin of 'antiperthitic' and 'pure' albite-oligoclase.*—These two feldspars have been treated together as they are genetically connected with each other and one grades into the other. They are of much later generation than the microcline perthites. This is borne out by the microscopic study of one or two specimens which show both the feldspars, pink microcline and white albite-oligoclase. A section cut at the contact of these two feldspars, reveals the following relation between them (Pl. XIV, Fig. 1):—

(1) Albite-oligoclase protrudes into the microcline and *vice versa*, but the protrusions of the former often have a step-like arrangement suggesting their guidance first by one and then by the other twin-plane of the microcline. The protrusions of the microcline do not show any such relation with the albite-type of twin-planes of the albite-oligoclase.

(2) The albite-oligoclase has no connection with the albite-veins of the microcline. These veins often do not meet the contact plane between their microcline host and the adjoining albite-oligoclase.

(3) Isolated 'islands' of microcline occur near the junction of the two feldspars. They are enclosed in the albite-oligoclase antiperthite and are in optical continuity with the main microcline but not with the patches of antiperthitic microcline present in the albite-oligoclase.

(4) Quartz occurs within both the types of feldspars and also near the contact between them. The quartz grains which are fully enclosed in albite-oligoclase are in optical continuity with some totally included in microcline perthite. Moreover, quartz shows a replacing relation to albite-oligoclase when seen under highest magnification, as its margin is occasionally very serrate and it sends out minute tongues into the plagioclase parallel to the twin planes. It is also replacing microcline, as it includes islands of microcline which are in optical continuity with the adjacent microcline.

From the above relations, it is definite that the 'antiperthitic' and the 'pure' albite-oligoclase feldspars are of later generation than the vein-perthites and that they are replacing the latter. It is also suggested from the peculiar behaviour of quartz inclusions in the two types of feldspars near their contact that the intergrowths, so often explained as due to simultaneous crystallisation, may be due to replacement.

Some of the specimens described under 'antiperthites' resemble the 'patch' perthites of Anderson, and the fact that this feldspar like his patch-perthites grades into the 'pure' albite-oligoclase may be given as an argument for naming the Kodarma antiperthites as 'patch' perthites. Patch perthites, however, are formed by extensive replacement of microcline by soda-feldspar,

and represent the next stage after vein-perthites. If the process of replacement were such a continuous one, as postulated by Anderson, we should expect to get some perthites giving intermediate values between vein-perthites containing about 20% albite and our 'antiperthite' containing about 95% albite-oligoclase. It is true that the proportions of the soda- and potash-felspars have been determined only in a few specimens of this area, but there does not seem to be in the whole collection, a single section of felspar which may approach the perthite of intermediate composition, say 50 volume percentage of each felspar. If the assumption is correct that such perthites do not exist in Kodarma, then it follows that there is some fundamental difference in the genesis of the 'vein' perthite and the felspar described here as 'antiperthite'.

Various authors have suggested that the pegmatitic magma splits into potash-rich and soda-rich fractions. Derry<sup>8</sup> put forward that view in 1931. The albitization of the 'simple' microcline-pegmatites, so often observed in the field, supports this view. Landes<sup>9</sup> believes that in the larger pegmatites the replacing solutions are the residual portions of the pegmatite magma itself. Spencer<sup>10</sup> has recently advanced a similar view that the granite-magma at about 800° C. in the presence of much water and free silica, splits gradually into a soda-rich and a potash-rich fraction. It is, therefore, suggested here that the "antiperthitic" and "pure" albite-oligoclase felspars have crystallised from solutions emanating from the soda-rich fraction of the pegmatitic magma at a later stage after the formation of the microcline perthite of the vein type. The patches of microcline seen in the antiperthitic soda-felspar might have been formed by the simultaneous crystallisation of the still remaining potash portion in that magma. There has been, however, intense resorption and corrosion of these patches of microcline, after their formation.

#### V. Conclusion.

The felspars of the pegmatites of Kodarma have been classified with special reference to their perthitic textures. It has been suggested that there have been at least two distinct periods in the evolution of the felspars of the pegmatites, the first is represented by orthoclase and microcline perthites of the composite 'vein' and 'film' type and the second by albite-oligoclase of 'antiperthitic' and 'pure' type. The former phase preceded the latter phase. The felspar of the second phase shows a replacing relation to that of the first phase when the two occur together. These two phases of the generation of felspars may be explained as due to the differentiation of the granitic residue into potash-rich and soda-rich fractions. The origin of the various types of perthitic intergrowths—'films', 'stringers', 'veins',

'inclusions' and 'antiperthitic' patches—have been discussed on the basis of their textural features, and the rôle of deuteric replacement has been emphasised in the formation of vein-perthites.

#### VI. Acknowledgements.

The author is grateful to Prof. H. H. Read of the Liverpool University, and to Prof. S. K. Roy of the Indian School of Mines, for their valuable suggestions during the study of these feldspars. He is thankful to his friend, Mr. G. C. Chattopadhyay, for his help in the preparation of the photomicrographs which illustrate this paper.

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#### EXPLANATION OF PLATES.

##### PLATE XII.

- FIG. 1.—'Stringers' and small patches of albite in orthoclase host. Patch (P) contains inclusions of muscovite and biotite. Opaque iron ore has been deposited mostly along a crack. Orthoclase perthite ( $K_1/8$ ) — .001 section  $\times 17$ ; between + nicols. Locality—S. of Budhwa-Asangarh (Sheet 150. N.W./1).
- FIG. 2.—Regular spindle-shaped 'veinlets' of albite showing 'en echelon' arrangement. Patch (P) of albite contains minute inclusions of opaque iron ore and a relict portion of orthoclase host. Same specimen ( $K_1/8$ ) as above — 010 section  $\times 60$ ; between + nicols.
- FIG. 3.—Twinned and untwinned lamellæ of microcline. The 'veins' (V) of albite are mostly confined to the twinned portions of microcline. Microcline perthite ( $K_3/22$ )  $\times 17$ ; between + nicols. Locality—Dipliswa stream (Sheet 149. S.W./1 & 2).

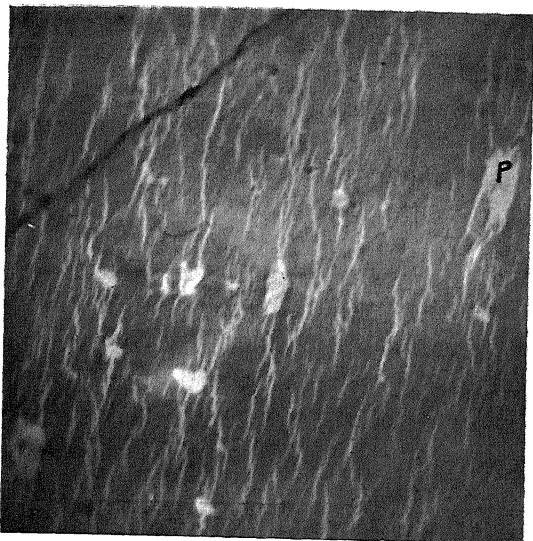


FIG. 1.

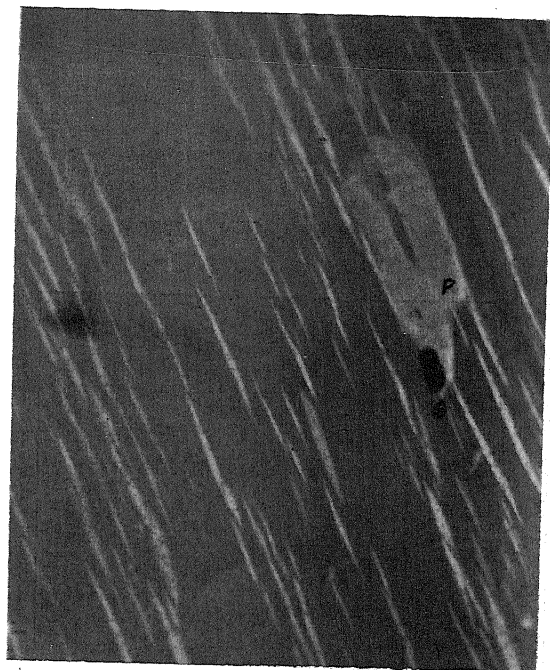


FIG. 2.



FIG. 3.



FIG. 1.

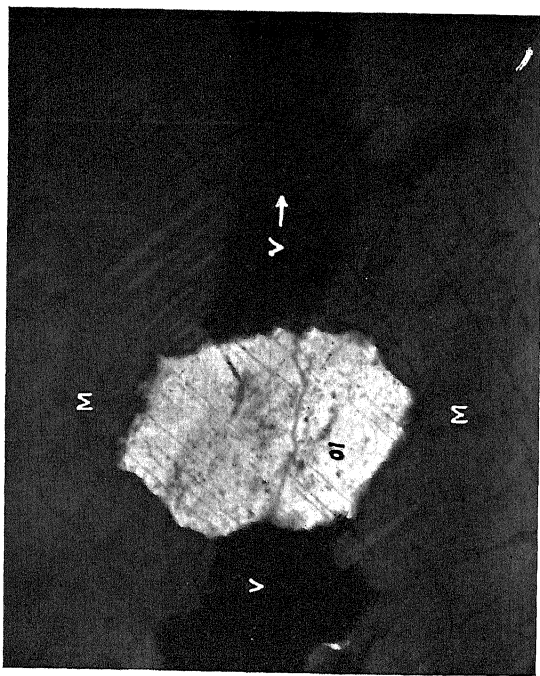


FIG. 2.

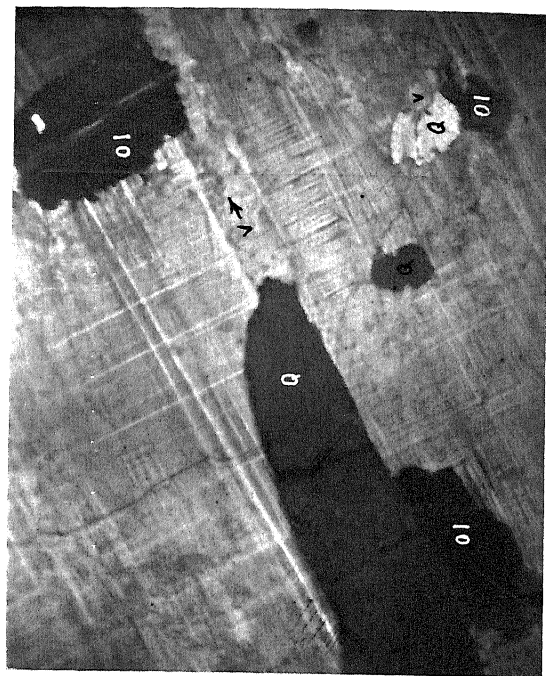


FIG. 3.

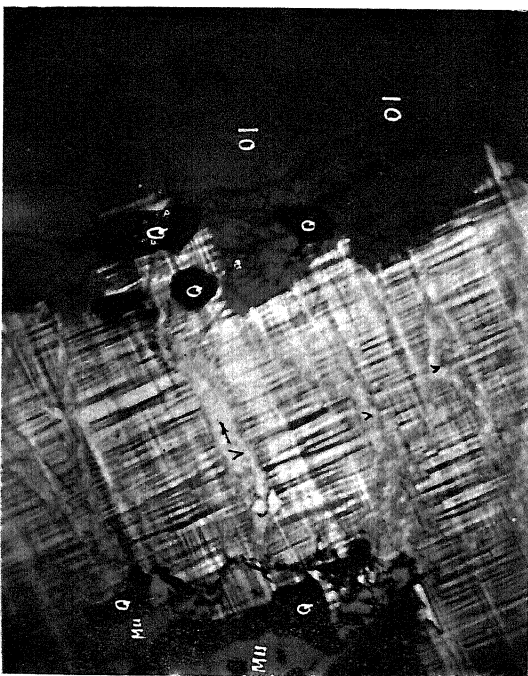


FIG. 1.

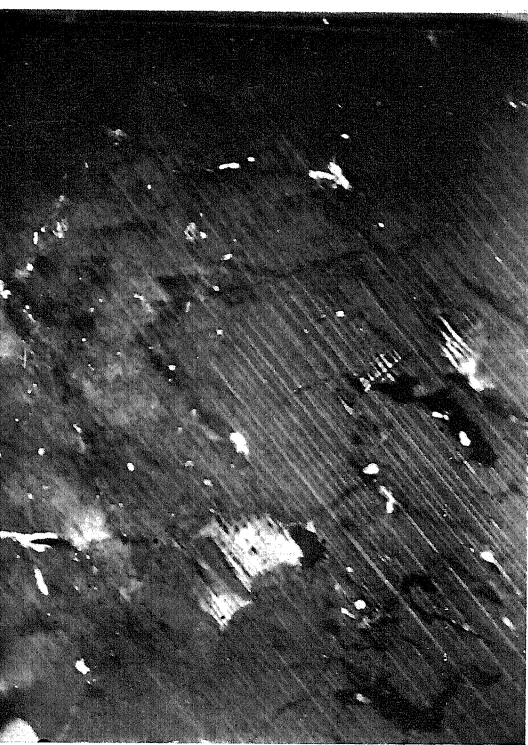


FIG. 2.



FIG. 3.



## PLATE XIII.

- FIG. 1.—'Films' of albite (shown as thin lines) between the two 'veins' (V) of albite in microcline host. Microcline perthite ( $K_2/117$ ) — .010 section  $\times 60$ ; between + nicols. Locality—E. of Sorangi Hill (Sheet 150. N.W./2).
- FIG. 2.—'Inclusion' of albite-oligoclase (ol) in microcline (M), cutting across the albite-vein (V). Microcline perthite [ $K_4/162(i)$ ]  $\times 60$ ; between + nicols. Locality—Sq. 39-Q (Sheet 149. S.W./4).
- FIG. 3.—Inclusions of albite-oligoclase (ol) and quartz (Q) in microcline with albite-veins (V). An oligoclase inclusion shows minute relict piece of vein-albite. Microcline perthite ( $K_4/202$ )  $\times 17$ ; between + nicols. Locality—Sq. 34-b (Sheet S.W./4).

## PLATE XIV.

- FIG. 1.—'Pure' and 'antiperthitic' albite-oligoclase (ol) showing replacing relation to microcline containing albite-veins (V) and inclusions of muscovite (Mu) and quartz (Q). Inclusions of quartz in microcline and those in albite-oligoclase are in optical continuity with one another. Junction of microcline perthite and albite-oligoclase ( $K_2/117$ )  $\times 17$ ; between + nicols. Locality—E. of Sorangi Hill (Sheet 150. N.W./2).
- FIG. 2.—'Antiperthitic' patches of microcline in albite-oligoclase. Long and rounded flakes of muscovite. Albite-oligoclase antiperthite ( $K_4/115$ )  $\times 17$ ; between + nicols. Locality—Jamuna mine (Sheet 149. S.W./4).
- FIG. 3.—'Chess-board'-like structure shown by albite-oligoclase of 'pure' and 'antiperthitic' types. Same specimens ( $K_4/115$ ) as above—another section  $\times 17$ ; between + nicols.

## TWO NEW SPECIES OF PACHYTROCTIDÆ (COPEOGNATHA) WITH A NOTE ON THE FAMILY.

BY RAMDAS MENON.

(Wilson College, Bombay.)

Received July 9, 1938.

(Communicated by Dr. S. C. Devadatta.)

### 1. *Psacadium georgi*, sp. nov.

(Fig. A ; Fig. 1, A—E.)

#### A.—Apterous form.

Several specimens (♀♀). Loc.—Tripunithura (Cochin State). Collected from dry leaves of *Musa paradisiaca* on 30.XII.1935.

Length of body.—1.5—1.7 mm.

Head more or less ovoid, posteriorly broader, with the hind angles well rounded: ferruginous brown; with scattered fine hairs which are more numerous towards the anterior region. Median epicranial suture present; frontal sutures wanting. Occipital margin more or less straight. Eyes small; 1.0.3:1\*; set very near the postero-lateral angles of the head; hemispherical; bare. Ocelli wanting. Antennæ comparatively very long, more than twice the length of the body; scape and pedicel sub-equal, short and bulbous, light yellowish brown; flagellar segments long and slender; the first six flagellar segments yellowish brown, the rest progressively less tinted and the segments towards the tip almost hyaline; the distal third of the third flagellar segment and the remaining ones with close-set annulations; the number of flagellar segments indeterminable owing to the joints being indistinguishable from the segmental annulations towards the tip of the antennæ. Maxillary palpi (Fig. 1, B) 4-jointed; 1st segment very short, 3rd slightly longer than this, 2nd twice as long and 4th about  $2\frac{1}{2}$  times as long as the 3rd; the last segment somewhat narrowed from the middle towards the tip; segments 2–4 with bristly hairs and yellowish brown in colour. Maxillary 'picks' (Fig. 1, D) bidentate, with an outer long and inner short tooth. Thorax ferruginous brown. Pronotum well developed; dorsally visible; undivided. Alinota fused together; the line of fusion

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Types of both species are at present kept with the author.

\* "1.0" is used after Pearman, J. V. (*Stylops*, 3, Pt. 6, p. 121) to signify the ratio between the inter-ocular space and the apparent eye-diameter as seen from the front of the head.

marked by a semicircular groove the convexity of which faces posteriorly; mesonotum about  $\frac{2}{3}$  the size of the metanotum; both the alinota not divided into the characteristic notal sclerites. Legs (Fig. 1, E) rather long and slender; trochanters with a pair of long, conspicuous hairs situated in a depression about its middle; hind tarsi in the ratio 15:3:4; ctenidia wanting; femora, tibiae and tarsi with fine hairs. Abdomen more or less fusiform; ferruginous brown, except for a transverse band running over segments 3-5, which is creamy yellow. The subgenital plate somewhat produced into a small triangular process set with short, fine hairs: the rest of its outer surface with moderately long hairs and the margin with four hairs conspicuously longer than the others situated on either side of the middle line (Fig. 1, C). Paraprocts without any sense-cushion.

These insects briskly run about when disturbed and they are easily mistaken for small ants.

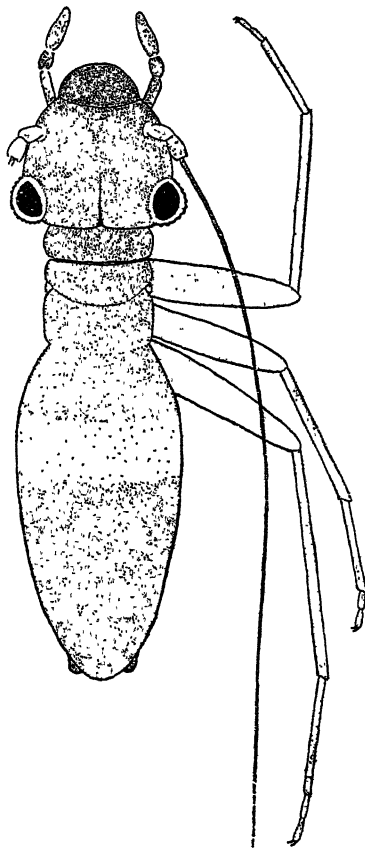
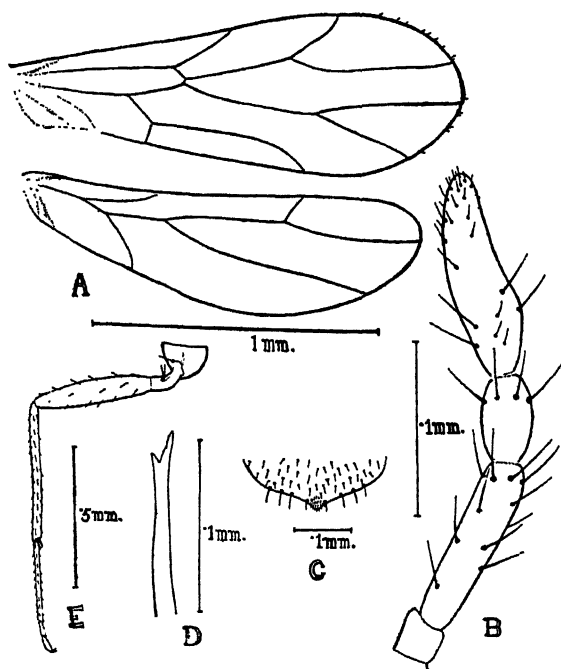


FIG. A.—*Psacadium georgi*, sp. nov. (apterous form).

FIG. 1. *Psacadium georgi*, sp. nov.

A.—Wings ; B.—Maxillary palp ; C.—Subgenital plate ; D.—Maxillary 'pick' ; E.—One of the meta-thoracic legs.

*B.—Alate form.*

4 Specimens (sex not determined). Collected along with the apterous form described above.

Length of body.—1.5 mm.

Length of forewings.—1.5 mm.

In their general characters these specimens agree well with the description given for the apterous form, except in the following details. Body lighter in colour. Alinota better developed ; divided by sutures to form the characteristic notal sclerites of winged psocids. Ocelli present ; three in number ; situated on the dorsal surface of the head ; somewhat wide apart to form a triangle ; more or less pinkish brown in colour. Wings well developed ; long and narrow, with rounded apices ; smoky in colour ; wing-membrane minutely punctate ; held flat one over the other parallel to the dorsal surface of the body as in the genus *Tapinella* Enderl. (1908). Fore and apical margins of the forewings with a few straight, short hairs rather at long intervals. Venation (Fig. 1, A) almost resembles

that of *Tapinella*; the distal segment of *sc* is absent; *rs* connected with *m* by a short cross-vein;  $r_{4+5}$  about  $1\frac{1}{2}$  times the pedicel of the radial forks; *m* only once forked, forks slightly shorter than the pedicel and more or less straight;  $cu_{1a}$  long and running almost parallel to the hind margin of the wings; areola postica long and narrow as in the genus *Tapinella*;  $cu_2$  united with  $m + cu_1$  at its base and its distal part only faintly demarcated; *la* present, free but fainter. Hindwings.—bases of *r*, *m* and  $cu_1$  fused together forming a stem vein from which the distal parts of these veins start off;  $r_1$  present;  $r_{4+5}$  almost as long as *rs*; *m* simple, comparatively long and more or less straight;  $cu_2$  and *la* free, but indistinctly demarcated.

The genus *Psacadium* was erected by Enderlein in the year 1908 for a species of apterous psocids collected from Takao (South Formosa). He named this species *P. bilimbatum*. Since that date no other species of *Psacadium* has been recorded. The Indian species described above can readily be distinguished from the genotype by its more or less uniform ferruginous brown colouration, with a transverse band of creamy yellow over segments 3–5 of the abdomen. *P. bilimbatum* Enderl. is whitish to ochraceous yellow, with broad brownish bands running longitudinally along either side of the body. The occurrence of alate form of *P. georgi*, sp. nov., perhaps indicate that winged form of *P. bilimbatum* may also be found.

I take great pleasure in naming the Indian species after Dr. C. J. George of Wilson College.

## 2. *Peritroctes cochinchensis*, sp. nov.

(Fig. B; Fig. 2, A–E.)

### A.—Apterous form.

7 specimens (♀♀). Loc.—Tripunithura (Cochin State). Collected from dry leaves heaped on the ground during April and December, 1935.

Length of body.—1.14 mm.

Head almost as broad as long and somewhat triangular; dark ferruginous brown, almost to brownish black. Occipital margin with a slight notch in the middle and the postero-lateral angles, behind the region of the eyes, produced posteriorly into blunt lobes (Fig. 2, C). Median epicranial suture present; frontal sutures wanting. Clypeus very tumid. Eyes small;  $1.0.3-3\frac{1}{2}:1$ ; somewhat morruliform; hemispherically protruding; with groups of rods between the facets arranged in the form of short combs. Antennæ 15-jointed; scape and pedicel sub-equal and somewhat bulbous; flagellar segments long and cylindrical, with close-set micrortichia arranged in the form of rings and a few scattered bristly hairs. Maxillary palpi (Figs. 2, D)

4-jointed ; 1st segment shortest, 3rd nearly twice as long as the 1st, 2nd and 4th segments about  $1\frac{1}{2}$  times the 3rd ; last segment somewhat narrowed from the middle towards the tip ; segments 2-4 with fine long hairs. Maxillary 'picks' (Fig. 2, B) tridentate ; outer tooth long, inner one short and the median one the shortest. Pronotum well developed and dorsally visible. Alinota almost the same size as the pronotum ; not divided by sutures to form the characteristic notal sclerites. Whole of the dorsal surface of the thorax lighter in colour than the remaining parts of the body ; creamy yellow. Legs long and slender as in the genus *Psacadium* ; coxæ, femora and the second and third tarsal segments of the fore and middle legs creamy yellow ; tibiæ and first tarsal segments ferruginous brown. Hind legs dark chocolate brown. Tarsi 3-jointed. Hind tarsi in the ratio 14 : 5 : 6. Ctenidia wanting. Claws more or less straight ; with a preapical tooth and a few microscopic fine hairs between this and the base. Abdomen sub-globular, with purplish brown mottles over an yellowish brown background. Subgenital plate (Fig. 2, E) with uniform-sized hairs on the outer surface and margin ; hind margin smoothly rounded ; its T-shaped chitinisation as figured. Whole of the body-surface with close-set sculpturing of the nature of small pits.

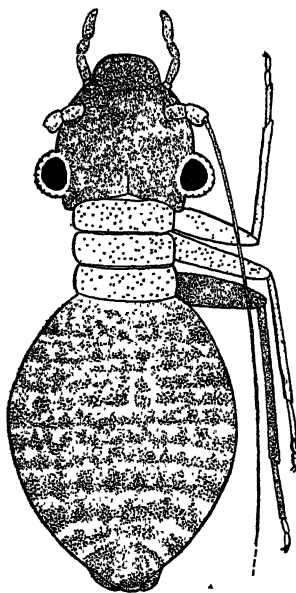


FIG. B.—*Peritroctes cochinensis*, sp. nov. (apterous form).

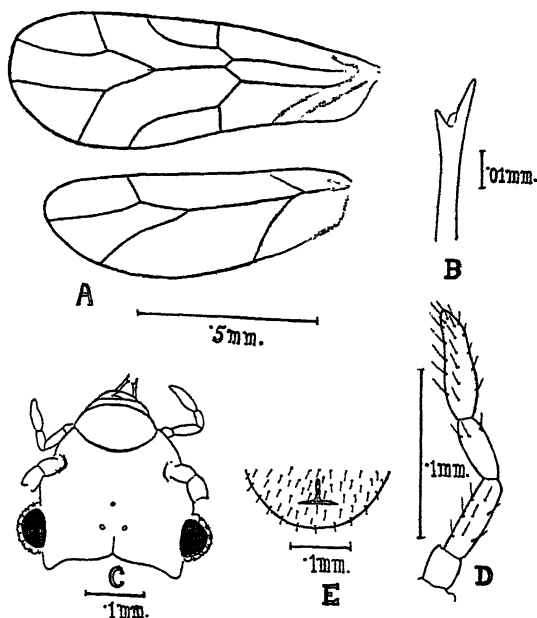


FIG. 2. *Peritroctes cochiniensis*, sp. nov.

A.—Wings; B.—Maxillary 'pick'; C.—Outline of the head (alate form); D.—Maxillary palp; E.—Subgenital plate.

*B.—Alate form.*

1 specimen (sex not determined). Loc.—Tripunithura (Cochin State). Collected along with the apterous form on 15.iv.1935.

Length of body.—1.07 mm.

Length of forewings.—1 mm.

The general characters of this tally well with the description of the apterous form except in the following details. Body somewhat lighter in colour. Alinota with the characteristic notal divisions of winged psocids. Ocelli present, three in number and situated on the dorsal surface of the head to form a small triangle; darker along their inner margins and whitish outside. Wings well developed; somewhat narrow; with rounded apices; folded flat one over the other parallel to the dorsal surface of the body as in the genera *Tapinella* and *Psacadium*. Wing venation (Fig. 2, A.) somewhat resembling those found in these genera. Forewings.—the distal segment of *sc* present and taking its origin from *r*<sub>1</sub>, *rs* and *m* fused together over a comparatively long stretch; *m* forked only once and the branches somewhat widely divergent; *cu*<sub>1a</sub> long, running parallel to the hind margin of the wing and with its tip smoothly curved; areola postica long and narrow; *cu*<sub>2</sub> fused

with  $m + cu_1$  at its base;  $1a$  free, but faintly demarcated. Hind wings.—bases of  $rs$ ,  $m$  and  $cu_1$  fused together to form a common stem vein from which the distal parts of these veins start off;  $r_1$  present;  $cu_2$  and  $1a$  free and only faintly demarcated.

The genus *Peritroctes* was proposed by Ribaga in the year 1911 to take an apterous species of psocids received from Natal (Africa). He named this species *P. natalensis*. Since its discovery no further records of *Peritroctes* have been made. The Indian species described here can readily be distinguished from the genotype by its darker colouration and the peculiar shape of the head. The presence of an alate form of this species shows that such a form may be found in *P. natalensis* Rib. also.

#### *A Note on the Family Pachytroctidæ.*

The occurrence of alate forms of *Psacadium* and *Peritroctes* throws better light on the homogeneous nature of the Family Pachytroctidæ created by Pearman by the amalgamation of Enderlein's Subfamilies Pachytroctinæ and Tapinellinæ. It may be remembered that when *Pachytroctes* was discovered, Enderlein (1905) placed it in a new subfamily, the Pachytroctinæ, and assigned it to the Family Troctidæ, which he recognized as a group of apterous psocids. In 1908 the same author discovered simultaneously the genera *Psacadium* and *Tapinella*, but ignoring the close morphological affinities between these two genera, he assigned the former (*Psacadium*) to the Pachytroctinæ and the latter (*Tapinella*) to a new subfamily, Tapinellinæ, which he included in an altogether different family, the Empheriidae. Enderlein's main reason for alienating *Tapinella* from the Pachytroctidæ was its possession of fully developed wings with venation somewhat resembling that of *Empheria*, the type genus of Empheriidae. Pearman, from his recent morphological studies, concluded that *Tapinella* is more allied to *Pachytroctes* than to *Empheria*, and he therefore, in his classification of psocids (1936), included *Tapinella* and *Pachytroctes* under one and the same family, the Pachytroctidæ. My discovery of alate specimens of *Psacadium*, with wings and venation very closely resembling those of *Tapinella*, proves conclusively that Pearman's classification is well founded. I may even go a step further and propose the entire suppression of one of the subfamilies, because the retention of both would mean the assignment of the apterous forms of *Psacadium* and *Peritroctes* to the Pachytroctinæ and their alate forms to the Tapinellinæ. Since I feel that perhaps winged *Pachytroctes* may be found and, moreover, this genus has priority of discovery over *Tapinella*, I would personally prefer the suppression of Tapinellinæ.

*Acknowledgements.*

Before concluding I wish to express my sincere thanks to Dr. C. J. George of Wilson College, Bombay, for kindly going through the manuscript and offering his valuable suggestions and criticisms and to Dr. Nathan Banks of the Museum of Comparative Zoology at Harvard College for his valuable opinion on my identification of these psocids.

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# THE EARLY LARVAL STAGES OF TWO SPECIES OF *PALÆMON*.

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Received July 9, 1938.

(Communicated by Prof. R. Gopala Aiyar, M.A., M.Sc.)

THE genus *Palæmon* includes a number of species, most of which are inhabitants of fresh-water. Some of them have comparatively large eggs and their larval life has been shown to be very much abbreviated. Others have much smaller eggs and these are believed to hatch out into the usual zoea larva characteristic of the group. Gurney (1938) has proved the occurrence of such a larva in the case of *Palæmon lar* by examining the developing eggs. Beyond this, little more is known about the development of this large group of prawns.

In the months of October and November of last year I was able to obtain egg-bearing females of two species of *Palæmon*, namely, *P. rudis* and *P. carcinus*, from the backwaters of this place. Of these, the occurrence of the latter species in these waters has already been noted by Henderson and Matthai (1910). Large numbers of the adults of *P. rudis* occur in the Chilka Lake in Orissa in September and November and their absence at other times led Kemp (1915) to assume that they migrate at this time into the fresh or very slightly brackish-water of the lake in order to hatch their eggs. One of the present species, namely *P. carcinus*, may apparently be a similar immigrant into the backwaters, since it is generally obtained only towards the close of the monsoon period (September–November) when the salinity is very low.

The specimens were kept alive in brackish-water and the small eggs readily hatched out into the first stage in a few days. Some of them moulted into the second stage in the course of the next two days; but the subsequent stages could not be secured in the same way since they perished rapidly after the moult. Due to lack of facilities it was not possible to look for them in the plankton. This paper therefore deals with only the first two stages; but even so it may be of some interest as nothing is known about them at present.

*Palæmon rudis* Heller.

Stage I. Length 1.75 mm. (Fig. 1).

The carapace has a long, slender rostrum which is more than half of the antennular peduncle in length. Its anterolateral angles are drawn out into

small, but clearly visible, pterygostomial spines. There is no dorsal papilla or spine. Abdominal segments are unarmed. Telson (Fig. 9) is considerably wider than long and its posterior margin is slightly concave and bears 7 spines on each side, the innermost spine being much shorter than the rest.

*Colouration*.—The animal is transparent. The oral region on the ventral side and the middle of the lateral portions of the carapace have narrow streaks of orange-red pigment. On the dorsal surface of the third abdominal somite there is a median elongated and slightly branching pink chromatophore flanked by two smaller yellow ones.

The appendages are very similar to those of *Brachycarpus* and *Leander*.

*Eyes*.—Eyes are sessile.

*Antennule* (Fig. 2).—Peduncle is unsegmented. Outer flagellum is an unsegmented papilla bearing at its tip 4 æsthetes and a short plumose seta. Inner flagellum is represented by a large plumose seta only.

*Antenna* (Fig. 3).—Peduncle bears a slender pointed spine opposite the base of the flagellum. The latter is finger-shaped, unsegmented and about two-thirds as long as the scale. Terminally it carries a long plumose seta and a spine. Scale is divided distally into four clearly marked joints. It carries along its inner margin and tip ten setæ of which the most distal is little more than a spine. Two short setæ are present on the outer margin of which the proximal is very small. Close to the proximal seta of the inner margin there is a small papilla.

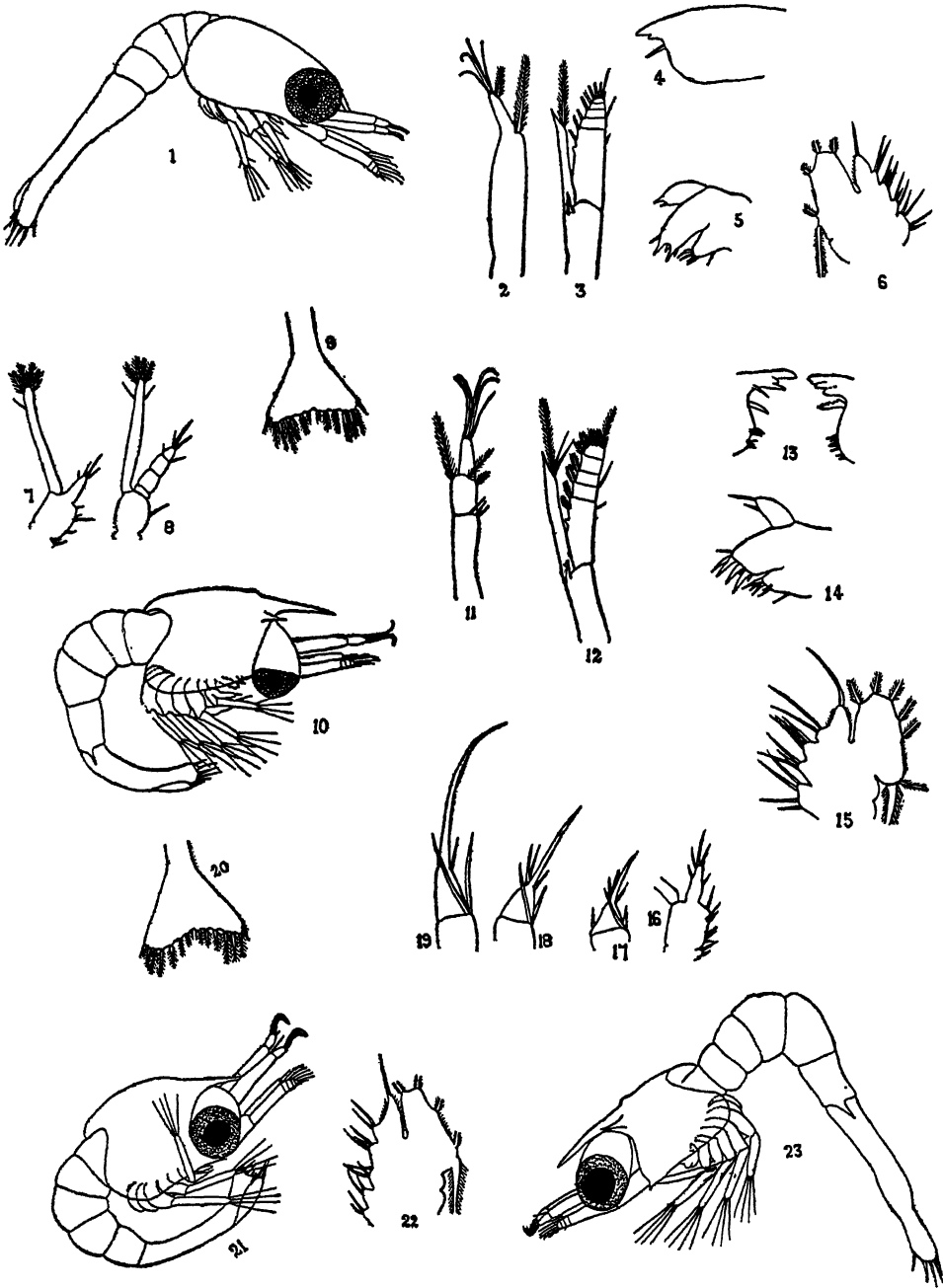
*Mandible* (Fig. 4).—Incisor part has one or two short blunt teeth. Molar portion is smooth or has 2–3 minute teeth. Between the two, but nearer the incisor part, there is a spine, which is slightly thicker on one side.

*Maxilla I* (Fig. 5).—Proximal masticatory process is narrow and is armed with 4 setæ at the tip and one at the middle of its proximal margin. Distal process has 4 teeth, 2 of which are much smaller than the others and a short seta. Palp is small and has two terminal spines, the proximal spine being smaller.

*Maxilla II* (Fig. 6).—There are 3 masticatory processes on the protopodite, of which the proximal is much larger than the others. It is armed with 4 and the others with 3 setæ. Endopodite has a basal lobe carrying 2 setæ and is tipped with a single seta. Scale has 5 plumose setæ along its margin, the hindermost of which is considerably larger than the rest.

*Maxilliped 1* (Fig. 7).—Coxopodite is reduced and has 2 very short setæ. Basipodite is only slightly produced inwards and carries 4 small setæ. Endopodite is an unsegmented process with 3 terminal setæ and a small one on

the outer margin. Exopodite has 4 plumose setae terminally and another small one on its hinder margin.



*Maxilliped 2* (Fig. 8).—Coxopodite is unarmed. Basipodite has a single seta. Endopodite consists of 4 segments of which the first is not so clearly marked off as the others. Third segment has one seta on its inner margin; last segment has three setæ of unequal size terminally and a small one at the base of the outer margin. Exopodite has 6 setæ, 4 large and 2 small ones.

*Maxilliped 3*.—This is similar to maxilliped 2, save for the presence of 2 setæ on the third segment of the endopodite.

Behind the third maxilliped there are large biramous rudiments of the first two peræopods.

*Stage II. Length 2 mm. (Fig. 10).*

Rostrum is more than two-thirds of the antennular peduncle in length. Besides the pterygostomial spine there is a large and prominent supraorbital spine on either side and just behind the base of the rostrum there is a small median papilla. Eyes are now stalked and freely moveable. First two pairs of peræopods are well-developed biramous appendages, having exopodites provided with natatory setæ. Abdominal somite 5 has a pair of lateral spines on its posterior margin and an additional pair of very small spines have developed in the posterior margin of the telson (Fig. 20) between the innermost pair of the previous stage.

*Colouration*.—The ventral surface and the distal end of the dorsal surface of the optic peduncles are tinged with yellow. A small yellow chromatophore has made its appearance at the base of the telson. Otherwise colouration is same as in the previous stage.

*Antennule* (Fig. 11).—The distal segment of peduncle is now clearly cut off. Three short setæ are borne by the proximal segment terminally and the distal segment has two more short setæ in addition to the large plumose seta of stage 1. Flagellum is still unsegmented and is tipped with 5 aesthetes, one of which is slender and pointed.

*Antenna* (Fig. 12).—Except for the appearance of two more slender setæ at the tip of the flagellum the antenna shows no alteration.

*Mandible* (Fig. 13).—The mandibles of the two sides differ slightly from one another. On the right side the incisor part has 3–4 short, blunt teeth and the molar portion has 6–8 very small spines. Between them there is a large spine, serrated on its ventral margin. The left mandible has two large teeth and a slender spine on the incisor part. The molar process is similar to that of the right mandible. Between them there is a slender spine which does not seem to be serrated.

*Maxilla 1* (Fig. 14).—Both endites have a few more spines and setæ. Palp is tipped with two setæ of which the proximal one is short and spine-like.

*Maxilla 2* (Fig. 15).—Only the scaphognathite shows some difference. It is now fringed with 7 plumose setæ.

*Maxilliped 1* (Fig. 16).—Basipodite has now 6 setæ. Endopodite is still unsegmented and armed with an additional seta on its inner margin. Exopodite is unaltered in all the three maxillipeds.

*Maxilliped 2*.—The third segment of the endopodite has now 2 setæ distally and the last joint also has one more seta terminally (Fig. 17).

*Maxilliped 3*.—Basipodite has now 2 setæ on its inner margin. Endopodite consists of 5 segments. The first two have each one seta on the inner margin, the third has none; the fourth has 2 and the last 4 terminally (Fig. 18).

*Peræopods 1 and 2*.—Coxopodite is reduced. Basipodite has two setæ as in maxilliped 3. Endopodite consists of 5 segments; the first two have one seta each, the third has one (on the outer margin) and the fourth has two. The last segment has only 2 setæ, one of which is small (Fig. 19). The larger seta on the terminal segment of the endopodite of the first peræopod is considerably bigger than the corresponding seta of the maxillipeds and of the second peræopod and the total length of the seta and the segment which carries it is equal to or slightly more than the rest of the endopodite. Exopodite is similar to those of maxillipeds 2 and 3.

Behind the second peræopod there are rudiments of two of the remaining appendages, the anterior one being biramous.

*Palæmon carcinus* Fabricius.

*Stage I. Length 2–2.25 mm. (Fig. 21).*

The larva is practically identical with Stage I of the previous species in appearance and structure of the appendages. It is, however, longer and more slender than the previous one and this, together with the colouration, are the only characters by which it could be distinguished.

The carapace has the rostrum and pterygostomial spines exactly as in the previous species. There is no dorsal papilla in this stage. The posterior margin of the telson is slightly concave and bears 7 pairs of spines.

*Colouration*.—The tips of the antennular peduncles and the oral region on the ventral side are beautifully pink in colour. A branching chromatophore of the same colour is present on the dorsal side of the third abdominal somite and another at the base of the telson on the ventral side.

The appendages are almost identical with those of the corresponding stage of the previous species so that it is not necessary to describe them again. In the second maxilla (Fig. 22) the proximal endite is much larger than the other two and is armed with four setæ as in the former species. But the two proximal setæ are separated, very often, from the distal ones by an indentation of the edge of the endite.

*Stage II. Length about 2.5 mm. (Fig. 23).*

The larva possesses all the characters noticed in the corresponding stage of *P. rudis*. In addition to the rostrum and pterygostomial spines the carapace bears a pair of prominent supraorbital spines and a median papilla behind the rostrum. Abdominal somite 5 has a pair of lateral spines and the posterior margin of the telson has now 8 pairs of spines, the innermost pair, as in the previous species, being extremely small. The first two pairs of legs are biramous with well-developed exopodites and endopodites armed with setæ in the same way as in Stage II of *P. rudis*. Eyes are stalked.

*Colouration.*—Besides the oral region and the distal end of the antennular peduncle, the posterior side of the base of the optic stalks and the proto-podites of the first two pairs of maxillipeds are also coloured pink. Abdominal segment 3 has now two large, branching chromatophores on the dorsal side, each of which sends off a branch into the second segment. The base of the antennal peduncle also has a small chromatophore. All the chromatophores are pink in colour.

The appendages are all similar to those of Stage II of *P. rudis* and do not need to be described. The peculiarity in the arrangement of setæ of the proximal endite of maxilla 2 was noticed in this stage also.

*Remarks.*—In a recent paper (1938) Gurney has reviewed our knowledge of the larvæ of the Palæmonidæ. Of the subfamily Palæmoninæ, larvæ of the two genera *Leander* and *Palæmonetes* are fully known. The first stage of a third genus *Brachycarpus* is also known. Besides these a number of unidentified larvæ belonging to the later stages have also been described under the name *Retrocaris* which, according to Gurney are, in all probability, the later stages of either *Palæmon* or *Brachycarpus*.

The fact of the existence of an ordinary zœa larva in the development of these prawns, which was hitherto only a matter of inference, is now confirmed and presumably it passes through all the stages recorded in the case of allied forms. From the preceding account it is also clear that, excepting differences in the armature of the carapace and abdominal somites, the larva in the early stages resembles closely the other members of the subfamily

mentioned above. In the absence of the later stages the interesting question of the identity of the *Retrocaris* larvæ still remains unsettled.

I am very grateful to Prof. R. Gopala Aiyar, Director of the Madras University Zoological Laboratory, for helping me with the necessary literature and to Prof. K. Karunakaran Nair, Head of the Natural Science Department of this College, for providing me with facilities for doing this work.

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(For explanation of figures see text.)

# ON *ECTEINASCIDIA BOMBAYENSIS* N. SP. (A NEW ASCIDIAN FROM BOMBAY.)

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### 1. Introduction.

THE present contribution is the first of a series in which the author desires to add to the existing knowledge of the ascidian fauna of the Indian coasts. Except for Herdman's work<sup>2</sup> in 1906, which deals with the fauna of the Gulf of Manaar, little systematic work on ascidians has been done in India. Ascidians are animals difficult to obtain and yet more difficult to preserve in an extended condition. The external form, shape and colouration of

preserved specimens are often misleading. The author has, therefore, laid more stress on internal characters as criteria for specific distinction.

The material was received in a preserved condition from the Royal Institute of Science, Bombay. The labels show that it was collected at Okha Port (Bombay) in 1926; but nothing is mentioned about the time of collection and the zone from which the material was collected. Three colonies of *Ecteinascidia* were received, one of which was quite well preserved, the other two being very much shrunk and ill-preserved.

My acknowledgements are due to Prof. P. R. Awati of Bombay for sending me the material, and to Prof. N. J. Berrill of Montreal for sending me his paper<sup>1</sup> on *Ecteinascidia*. To Prof. K. N. Bahl of Lucknow I am indebted for giving me the necessary facilities for work and for kindly reading through the manuscript.

## 2. External Characters.

The colony consists of ten to twelve individuals, each being attached by a short thin peduncle (Plate XV) to a basal stolon network. The stolon is not constricted but forms an anastomosed pattern (Fig. 3). Each individual is cylindrical, wider at the free end than at the middle or the fixed end (Figs. 1 and 2). The size varies slightly from one individual to

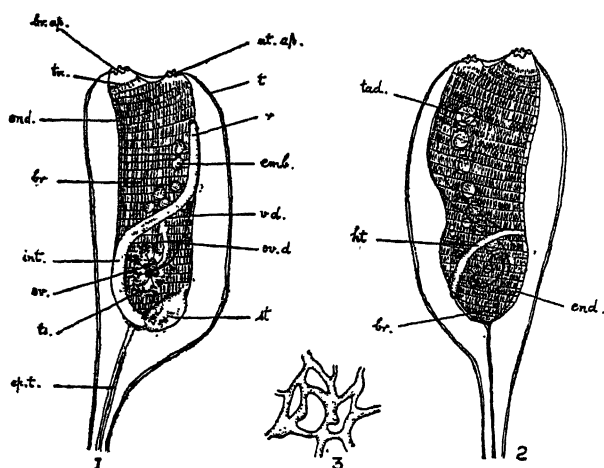


FIG. 1.—Mature individual of *Ecteinascidia bombayensis* from the left side ( $\times 4$ ).

FIG. 2.—Mature individual, from the right side ( $\times 4$ ).

FIG. 3.—Stolon ( $\times 4$ ).

another. The maximum size measured was 23 mm. and the minimum 18 mm., while the maximum thickness of an individual was 7 mm. The colour of the preserved specimens is dirty white, but this is certainly due to

the effect of the preservative. There is no differentiation of the body into thorax and abdomen. The branchial and atrial siphons are of moderate size, the branchial being a little larger than the atrial. The apertures are comparatively far apart, each being bounded by five lobes of the test.

### 3. The Test.

The test is thin and almost transparent allowing almost all the internal organs to be seen through it. There are no blood-vessels in the test but quite a large number of large bladder-cells are present.

### 4. Internal Organs.

(a) *Mantle*.—The mantle is thin and translucent and covers all the organs in the body. There are a large number of transverse anastomosing muscle-fibres arranged transversely in annular bands around the body. These muscles are present throughout the body but are weak at the posterior end of the animal and are totally absent over the intestine and the endostyle.

(b) *Branchial Sac*.—The branchial sac occupies nearly the whole length of the individual except the stalk through which the "epicardiac tube" passes. The transverse vessels are narrow and are all alike. The internal longitudinal bars are much narrower than the transverse vessels and are supported by wide cylindrical connecting ducts at the place of their junction with the transverse vessels (Figs. 7 and 8). There are no papillæ, unless

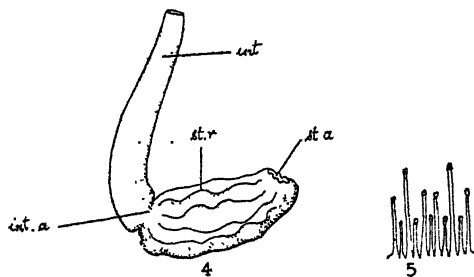
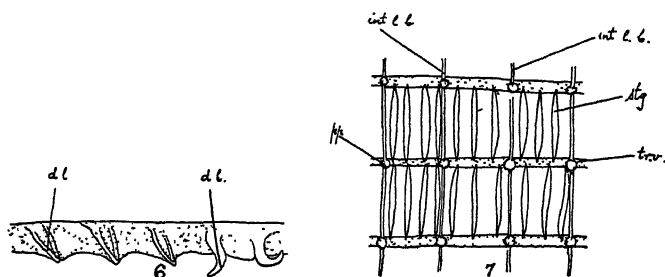


FIG. 4.—Side view of stomach and part of intestine ( $\times 20$ ).

FIG. 5.—Tentacles ( $\times 40$ ).

the projections beyond the internal longitudinal bars are considered as such. The stigmata are longitudinal in axis and regularly arranged, 3—4 to a mesh. There are about 40 stigmata in a single row.

(c) *Tentacles*.—The tentacles are all simple and are in three alternating sizes (Fig. 5). They are about 64 in number, the ventral ones being the longest. The tips of the tentacles are slightly swollen and appear transparent even when stained.

FIG. 6.—Posterior part of dorsal lamina ( $\times 60$ ).FIG. 7.—Wall of branchial sac enlarged ( $\times 40$ ).

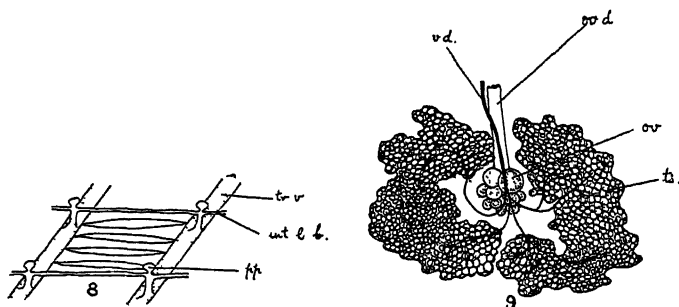
(d) *Dorsal Lamina*.—The dorsal lamina is in the form of a thin flap with wavy ridges on it and is provided with short triangular languets connected at their bases by a narrow membrane (Fig. 6). Two or three of the posteriormost languets are free and elongated.

(e) *Dorsal Tubercle*.—A small and simple rounded opening with raised walls represents the dorsal tubercle.

(f) *Alimentary Canal*.—The viscera project slightly beyond the pharynx at the posterior end of the animal. The stomach, placed almost at the posterior end of the animal, is a pear-shaped sac. It has slight longitudinal folds (Fig. 4) on its walls. The intestine is narrow and there is a moderate constriction between the stomach and the intestine. The intestinal loop is formed on the left side of the branchial sac. The rectal aperture is bounded by two large thick lips.

(g) *Heart*.—The heart is a thin-walled tube of sufficiently large diameter, bent like a crescent and situated on the right side of the body (Fig. 2).

(h) *Gonads*.—The gonads are present in the loop of the intestine, the testis-lobes being arranged in the form of a crescent around the centrally placed ovary. The testis-lobes consist as usual of a large number of testicular follicles (Fig. 9). The ovary is comparatively small consisting of ova

FIG. 8.—Wall of branchial sac enlarged ( $\times 40$ ).FIG. 9.—The gonads with part of their ducts ( $\times 10$ ).

of different ages. The vas deferens is formed by the junction of four or five spermatic tubes. It runs for some distance alongside the comparatively short and thick oviduct and opens alongside the rectum into the atrial cavity.

#### 5. Development.

Development is internal and takes place first in the oviduct and then in the atrial cavity which acts as a brood-pouch. Segmentation of the egg begins in the oviduct and when discharged into the atrial cavity it is already highly segmented. As development proceeds further the embryo passes towards the atrial aperture and more embryos take its place. Thus, a progressive series of developing embryos may be seen lying between the oviducal aperture and the base of the atrial siphon (Fig. 2), where the embryo is in the tadpole stage. Eight to ten embryos may be observed at a time in the same individual.

#### 6. Generic Characters.<sup>3</sup>

*Body* elongated with a short peduncle, but not divided into thorax and abdomen; *Test* thin and membranous, containing no blood-vessels; *Mantle* thin, musculature consisting of transverse bands; *Branchial sac* with internal longitudinal bars but no papillæ; *Dorsal lamina* represented by a series of tentacular languets, some being connected at their bases by a narrow membrane. *Tentacles* simple; *Viscera* on the left side of the branchial sac; *Gonads* placed in the intestinal loop the spermatic vesicles forming a crescentic curve around the centrally situated ovary.

#### 7. Specific Characters.

The individuals are large and their number in one colony is about twelve. Both the branchial and atrial apertures are five-lobed; the tentacles are in three lengths and number about 64; the languets in the posterior region of the dorsal lamina are conical long and free, while in the anterior region they are short and triangular and joined by a membrane. There are about 40 stigmata in a row and the internal longitudinal bars are supported by cylindrical ducts that connect them with the transverse vessels. There is a swelling on the top of each junction of the internal longitudinal bars with the transverse vessels, and this on first sight appears to be a papilla (Fig. 8). True papillæ are, however, absent. The stomach, which is usually plain in *Ecteinascidia*, has light longitudinal folds in the present species.

#### 8. Remarks.

The genus *Ecteinascidia* was established by Herdman in 1880. In 1891<sup>3</sup> Herdman himself described four species of the genus, viz., *E. diaphanis*, *E. moorei*, *E. turbinata* and *E. thurstoni*. To this list nine more species have been added, viz., *E. garstangi*, *E. euphues*, *E. psammodes*, *E. nexa*, *E. multiclathrata*,

*E. sluiteri*, *E. diligens*, *E. solida* and *E. conklini*. The present species *E. bombayensis* n. sp. resembles *E. solida* in its size and in its connecting ducts of the internal longitudinal bars; while its thin and transparent test, and slight folds on the wall of the stomach, brings the species close to *E. sluiteri*. It has 3 to 4 stigmata and about 60 tentacles in common with *E. thurstoni*.

*Ecteinascidia* appears to be a tropical type of ascidian structure, occurring so far as we know at present, only between Bermuda to the north and the north coast of Australia to the south; and having its main development in the eastern seas. Out of the fourteen known species ten occur in the Indian Ocean and Malayan seas. Of these again *E. sluiteri*, *E. thurstoni* and *E. solida* are known from Ceylon; while *E. bombayensis*, procured from Bombay, should be common on the West Coast of India.

### 9. Summary.

The author describes a new species of *Ecteinascidia* (fam. Clavelinidæ) collected from Bombay. The present species can be distinguished from other existing species by the following characters: the siphonal apertures are 5-lobed; tentacles are of three lengths and about 64 in number; the posterior dorsal languets are long conical and distinct; the transverse muscles completely encircle the pharynx; the internal longitudinal bars have projections comparable to papillæ; the stomach has folds in its walls.

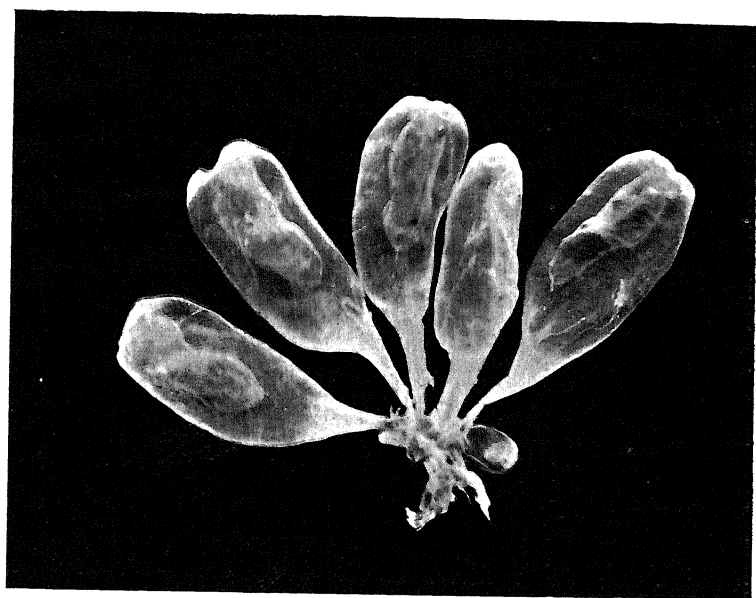
Ten to twelve individuals form a colony, each individual being attached by a peduncle to a basal stolon network. Development is internal, the atrial cavity acting as a brood-pouch, and the young are discharged into the sea as fully developed tadpoles.

### LETTERING OF ILLUSTRATIONS.

<i>at.ap.</i>	Atrial aperture.	<i>ov.d.</i>	Oviduct.
<i>br.</i>	Branchial sac (pharynx).	<i>pp.</i>	Papillary projection.
<i>br.ap.</i>	Branchial aperture.	<i>r.</i>	Rectum.
<i>d.l.</i>	Dorsal languet.	<i>st.</i>	Stomach.
<i>emb.</i>	Embryo.	<i>st.a.</i>	Stomach opening.
<i>end.</i>	Endostyle.	<i>st.r.</i>	Ridges on stomach wall.
<i>ep.t.</i>	Epicardial tube.	<i>stg.</i>	Stigmata.
<i>ht.</i>	Heart.	<i>t.</i>	Testis.
<i>int.</i>	Intestine.	<i>tad.</i>	Tadpole.
<i>int.a.</i>	Intestinal opening.	<i>tr.v.</i>	Transverse vessels.
<i>int.lb.</i>	Internal longitudinal bars.	<i>v.d.</i>	Vas deferens.
<i>ov.</i>	Ovary.		

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# PHOTOSYNTHETIC SPECIFICITY IN RELATION TO BIOCHEMIC CONSTITUTION OF LEAVES

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## *The Outlook*

ON surveying the flora of a definite locality, it becomes apparent that in spite of identical environmental conditions different species and varieties growing therein exhibit marked variations as to their general growth and ultimate dry matter yield. Not only are these variations to be observed from species to species and varieties to varieties but also within individuals of the same variety. Brown and Escombe,<sup>3</sup> Warburg and Negelein,<sup>12</sup> Lubimenko,<sup>6</sup> Plester,<sup>8</sup> Henrici,<sup>4</sup> and Willstätter and Stoll<sup>13</sup>, have shown on the basis of their manifold observations that such variations from plant to plant are due to differences in specific photosynthetic rate. The deviations in the rate of assimilation from one plant to another depend according to these investigators upon a number of factors the chief amongst which are the intensity of the external variables under which the plant undergoes development, the previous history of the plant, the number of stomata and the chlorophyll content.

Obviously chlorophyll being the most important it was questioned whether the differences in specific photosynthetic efficiency of leaves under identical external conditions were due to variations in the characteristics of the chloroplasts or the relative concentration of chlorophyll in different genera and species. Analysing this aspect in detail Willstätter and Stoll<sup>13</sup> clearly demonstrated the extraordinary uniformity of the chlorophyll pigments throughout the higher plants and in view of the photosynthetic number being not constant concluded that variations in assimilation rate are due to causes other than the chlorophyll content. Blackman and Matthæi<sup>2</sup> have shown that leaves in general have equal coefficients of economy in the photosynthetic process, and that the fundamental specific differences would seem to lie in their different coefficients of acceleration of activity with increase in temperature. Blackman<sup>1</sup> also opines that in view of the identity of the chlorophyll pigments throughout the plant kingdom, the first product of photo-reduction may also be identical leading one to believe that all plants

should have initially similar carbohydrate flux, the final products formed by condensation or otherwise being the result of the chemical configuration of the protoplasmic agents in contact with which the reactions go on.

The point at issue therefore, is whether the carbohydrate metabolism which is supposed to be identical at least till the first stage in the up-grade chain of reactions, has any bearing on the transformations inside the assimilating leaf. If so, whether the synthesised organic materials subsequently accumulating within the leaf bear any relation to the biochemic characteristics of the species on the one hand and the specific photosynthetic rate, on the other. The data obtained in this connection are presented in the following pages.

### *Experimentation*

In order to supply materials for experimentation, plants of varied biochemic constitution such as *Saccharum officinarum*, *Oryza sativa*, *Phaseolus* sp., *Ricinus communis*, *Triticum vulgare*, *Linum usitatissimum*, *Pisum sativum*, *Hordeum vulgare*, *Brassica alba*, *Allium cepa* and *Carthamus tinctorius* were grown on the Experimental Farms at this Research Station under conditions such that the soil nutrition was not limiting. Forty-five days after germination, during the adolescent stage of the life-cycle, the first series of experiments were conducted, no necessity being felt of recording such observations prior to this stage owing to the complications introduced by other factors during the early stages when the photosynthetic machinery and other interrelated plant processes are in a state of development.<sup>9</sup>

The experimental leaves were picked up a day previous to experimentation and kept under conditions similar to the one under investigation. Their rates of assimilation were subsequently estimated under optimum conditions of external variables by the continuous current method as detailed elsewhere.<sup>10</sup> Chlorophyll content of the material was estimated by Oltman's method.<sup>7</sup> The materials for chemical analysis were prepared after Link and Tottingham.<sup>5</sup> For analytic details the procedure and methods recommended by the *Official and Tentative Methods of Analysis*<sup>11</sup> were rigidly followed.

### *Results*

A survey of the data portrayed in Table I indicates that mature leaves gathered from plants of so different constitutions as *Linum usitatissimum*, *Triticum vulgare*, *Allium cepa*, *Pisum sativum*, *Saccharum officinarum*, *Oryza sativa* and *Phaseolus* sp. assimilates 1.96, 6.10, 8.22, 5.57, 7.85, 6.78 and 5.92 mgm. respectively during the early stage and 4.81, 17.6, 24.12, 12.75, 21.02, 18.95 and 13.12 mgm. of carbon dioxide during the later

TABLE I

Real assimilation in mgm. of carbon dioxide per hour per 100 sq. cm.  
of mature leaves at two stages of maturity.

Temperature—30.0° C. CO<sub>2</sub> concentration—0.3 per cent.

Illumination—1500 C.P. Phillips  $\frac{1}{2}$  watt lamp at 6 cm. distance.

Species	Real Assimilation	
	45 Days	85 Days
<i>Hordeum vulgare</i> .. ..	6.27	18.50
<i>Triticum vulgare</i> .. ..	6.10	17.60
<i>Brassica alba</i> .. ..	2.35	6.83
<i>Carthamus tinctorius</i> .. ..	5.43	9.33
<i>Pisum sativum</i> .. ..	5.57	12.75
<i>Linum usitatissimum</i> .. ..	1.96	4.81
<i>Allium cepa</i> .. ..	8.22	24.12
<i>Saccharum officinarum</i> .. ..	7.85	21.02
<i>Oryza sativa</i> .. ..	6.78	18.95
<i>Ricinus communis</i> .. ..	3.45	8.72
<i>Phaseolus</i> sp. .. ..	5.92	13.12

periods of the life-cycle. The rate of photosynthesis thus varies with the species under investigation, the variations in specific photosynthetic rates being more prominent during later stages of plant growth when due to age-developmental variations, the assimilating system is capable of a higher rate of assimilation even under identical conditions of external variables.

Since the conditions of the experiment were such that the external factors—light, carbon dioxide and temperature—were supplied in sufficiently high factor-intensity as not to ordinarily limit photosynthesis, the cause for the observed differences in assimilation rate must be sought in some internal factor governing the photosynthetic reaction. Of all such internal variables, the water-content (Table II) of the experimental leaves is found to deviate

TABLE II

*Water and chlorophyll contents and assimilation rate/chlorophyll content of mature leaves from eighty-five days old plants*

Species	Water-content percentage	Chlorophyll content mg.	Assimilation rate
			chlorophyll
<i>Hordeum vulgare</i> .. ..	74.8	3.5	5.2
<i>Linum usitatissimum</i> ..	79.6	3.4	1.4
<i>Pisum sativum</i> .. ..	78.1	3.5	3.6
<i>Allium cepa</i> .. ..	91.1	4.4	5.5
<i>Triticum vulgare</i> .. ..	71.0	3.4	5.1
<i>Brassica alba</i> .. ..	87.3	4.0	1.7
<i>Carthamus tinctorius</i> ..	82.5	3.8	2.4
<i>Saccharum officinarum</i> ..	73.2	3.5	6.0
<i>Oryza sativa</i> .. ..	82.4	3.9	4.9
<i>Ricinus communis</i> .. ..	72.8	3.4	2.5
<i>Phaseolus</i> sp. .. ..	80.0	3.3	3.9

quite disproportionately to the respective assimilation rates, except in the case of *Allium cepa* where the high photosynthetic rate seems to be correlated with the high water-content. The variations in specific photosynthetic rates in all cases thus cannot be accounted for on the basis of the water-content of experimental leaves. The causal factors must, therefore, be sought elsewhere.

The chlorophyll content of the leaf may be examined as the next factor governing the rate of assimilation. The plants providing the leaf material being mature specially towards the second period of observation, *viz.*, 85 days in the life-cycle, chlorophyll content as a matter of fact should have attained its maximum level possibly beyond the limiting value in each of the cases under investigation. Yet to have a direct insight into the respective chlorophyll content, chlorophyll extracts of the experimental leaves were obtained and analysed with respect to the chlorophyll contained in

them (*cf.* for method Oltman, 1933). That variations in chlorophyll content from species to species do not follow strictly the deviations in assimilation rate is shown by the varying values obtained for assimilation rate per unit chlorophyll content for the different species under investigation (Table II).

An analysis of the data further reveals that the plants under investigation show individual differences in the percentage of different organic materials accumulating within the assimilating leaves. Thus *Saccharum officinarum* leaves accumulate sugars to a greater extent as compared to starches, proteins and fats (Table III). *Oryza sativa*, *Phaseolus* sp., and *Ricinus communis* economise starches, proteins and fats respectively

TABLE III

*Glucose, Sucrose, Starch, Protein and Fat percentages (dry wt. basis) after a period of rapid assimilation in detached leaves*

Species	Glucose	Sucrose	Starch	Protein	Fat
<i>Saccharum officinarum</i> ..	2.8	18.2	14.4	13.0	6.5
<i>Oryza sativa</i> ..	2.4	6.9	22.7	13.1	5.6
<i>Phaseolus</i> sp. ..	2.0	4.7	9.1	24.8	8.5
<i>Ricinus communis</i> ..	1.6	4.0	8.0	10.2	14.4

more than other products. Such an increase in the percentage of one group of substances as compared to others, seems to be also correlated with the nature of the specific biochemic products that these very species accumulate towards the end of their life-cycle. *Saccharum officinarum* economising more of sugars during photosynthesis stores such simple carbohydrates in the stem towards the close of the life cycle in greater quantities as compared to other products and consequently such plants are grouped as sugary ones. The first indication of storage of sugars in far excess of other materials is thus found in the assimilating leaf. Similarly *Oryza*, *Phaseolus* and *Ricinus* having starch, protein and fat as their characteristic storage products respectively also show indications of the economy of such substances in leaves exposed to illumination long before the storage organs gave indication to this fact.

On segregating the experimental plants into different biochemic groups, according to the nature of the products accumulating in their tissues it

appears that each class differs from the other markedly in the range of assimilation (Table IV). Plants belonging to sugary constitution have

TABLE IV

*Range of photosynthesis in mature plants of varied biochemic constitution*

Species			Biochemic group	Range of assimilation in mgm. of CO <sub>2</sub>
<i>Allium cepa</i>	}	.. ..	Sugary	21-24
<i>Saccharum officinarum</i>				
<i>Oryza sativa</i>	}	.. ..	Starchy	17-18
<i>Triticum vulgare</i>				
<i>Hordeum vulgare</i>				
<i>Pisum sativum</i>	}	.. ..	Proteinaceous	12-14
<i>Phaseolus</i> sp.				
<i>Linum usitatissimum</i>	}	.. ..	Fatty	4-9
<i>Brassica alba</i>				
<i>Carthamus tinctorius</i>				
<i>Ricinus communis</i>				

apparently a higher rate of assimilation as compared to starchy, proteinaceous and fatty ones. The photosynthetic efficiency is thus related to the nature of the storage products, the simpler the biochemic product, the higher is the assimilation rate. In the light of these observations it appears therefore that although water and chlorophyll content do not show any definite relation with the varying photosynthetic rates, the variations from plant to plant under identical environmental conditions could be well explained in terms of their biochemic constitution.

#### Summary

An attempt has been made in this paper to study the photosynthetic activity of a number of plant species under optimum yet identical conditions of factor-intensity and to trace the relationship, if any, between the organic materials subsequently formed and the assimilatory influx of carbon dioxide.

Different plant species have different photosynthetic rates, the range of variation from species to species becoming more and more pronounced with advance in age of plants. Such variations under otherwise constant external conditions are discussed with special reference to internal factors.

The water-content of the experimental material no doubt differs from plant to plant but does not follow the same gradation as that maintained by assimilation rate. The chlorophyll content too shows no correlation with the assimilatory efficiency.

There seems to be a fair degree of correlation between the products economised in leaves during their photosynthetic activity and the products stored by the same species towards the close of the life-cycle. Plants economising sugars, proteins, starches and fats in assimilating leaves also store such substances respectively in their storage organs towards the close of their life-cycle.

The photosynthetic rate seems to be related to the nature of the end products accumulating in assimilating leaves. Leaves accumulating simpler sugars have fairly high rates of assimilation while those economising the more complex starches, proteins and fats respectively have to their credit decreasing assimilatory efficiency.

The biochemic constitution of the experimental material as judged by the products economised during assimilation appears to be an important internal factor governing photosynthesis and suggestively explains the phenomenon of photosynthetic specificity in certain groups of crop plants.

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## A 'TINY' SORGHUM.

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SORGHUM is the Great Millet. It is a giant among cereals and is the tallest of them. It is explained that this crop owes its name to the Italian word *Sorgo* (to rise), indicative of its rising head and shoulders over every other cereal. The height of sorghum has, therefore, attracted the attention of workers on this crop.

Conner and Karper (1927) while pursuing hybrid vigour in sorghum studied inter-varietal and intra-varietal crosses between different height groups in feterita, kafir and milo. "The three inter-varietal crosses made were characterised by marked hybrid vigour both in  $F_1$  and  $F_2$ , but the intra-varietal crosses showed no hybrid vigour. Crosses between Extra Dwarf milo and Standard milo gave the  $F_1$  which was intermediate between the parental heights, and the  $F_2$  distribution ranged from one extreme to the other. Extra Dwarf milo crossed with Dwarf milo showed increased height of plant in  $F_1$ . The difference between these two varieties was considered to be due to several genes, the increased height in the hybrid being probably an expression of two or more complementary genes for height. Crosses between Extra Dwarf feterita and Standard feterita displayed a monogenic difference between the parents, the tall variety dominating." (Matsuura, 1933.) Later Karper (1932) obtained "tall" mutants in kafir as hybrids which gave simple segregations for tall and normal in the  $F_2$ , tall being dominant. The increase in height was considered to be entirely due to the elongation of the internodes and not due to increase in the number of nodes. Sieglinger (1932) reports that in crosses between Standard broom-corn and Western Dwarf or Whisk Dwarf, the  $F_1$  was like the Standard parent, and the  $F_2$  gave a simple monogenic segregation for Standard and Dwarf. But in a cross between the two Dwarf types, the  $F_1$  was of the Standard type and the  $F_2$  gave a di-hybrid segregation of 9 Standard to 3 Western Dwarf to 3 Whisk Dwarf to 1 Double Dwarf. Sieglinger (1933) obtained also a monohybrid segregation for normal and dwarf plants in the  $F_4$  generation of a cross

between Red kafir and Reed kafir. The dwarf type is considered as resulting from a mutation due to the loss of a single height factor from the normal. Except in height and head length, the normal and dwarf plants were very similar.

The senior author and his co-workers (Rangaswami Ayyangar, *et al.*, 1937) pursued the inheritance of the character composite "short-early" and "tall-late" and note the differences to be due to internodal number and disposition in length. In the short group they find about 10 internodes with a unimodal disposition in length from the base upwards and in the tall group about 17 internodes with a bimodal disposition in length. They have reduced the height of the plant to its components, namely the internodes and their disposition in the plant. In a later publication, the senior author and his co-workers (Rangaswami Ayyangar, *et al.*, 1938) have analysed many varieties of sorghum and find that they fall into the three groups of internodal disposition, namely *steady increase*, *unimodal* and *bimodal*. They notice a parallelism in the disposition of the corresponding leaf-sheaths. The factors determining internodal length seem to operate within the framework of the three dispositions detailed above, there being a fairly wide range in the total height of the plant within these three dispositions.

The previous workers have symbolised the general phenomenon in which tallness was generally dominant to dwarfs and dwarfs to double dwarfs, etc., by giving the genetic symbols T and D indicative of tallness and dwarfness. A study of a wide range of sorghums made at the Millets Breeding Station, Coimbatore, shows, as will be seen from Fig. 1, that the sorghum varieties range themselves into a complete gradation in height. At the tall end is the tall and elegant *Sorghum elegans* from Tanganyika and at the dwarf end is the two foot milo from New Mexico, U.S.A. The use of such expressions as tall and dwarf and their abbreviations in genetics can only lead to a confusion in the long run. It seems to be necessary to recognise that the nett height is the ultimate expression of various internodal components, and any genetic symbolisation is best in terms of the symbol *In* for internode.

The work reviewed above is on normal, healthy grain sorghums under cultivation, picked and perpetuated in special localities for a special purpose like harvesting with a combine, etc. In the virgin interior of the tropics the primitive races could afford to grow varieties that could be cut with manual labour. Their introduction into America would naturally have stimulated mutational tendencies until the dwarfs that appeared are picked and have given the double dwarfs, etc., that are the wonder of the American sorghums.

In the exhaustive review of the genetics of sorghum published by J. H. Martin (1936) in the *Year-Book of Agriculture* of the United States of America, there occurs a reference to a midget sorghum that has proved a simple recessive to the normal height of kafir. The authors of this article have not had an occasion to know the details about this occurrence.

In this article, the occurrence and inheritance of a Tiny Sorghum in this great giant millet is recorded. In the year 1926 seeds of some Chinese sorghums were got down from Manchuria. One of these samples was M.S. 741, named Hei Ko She Jenhung. This was sown in the same year and a type plant from it was breeding true to type through the years 1927-35. The type had glumes that were elliptic, with long nerves and with long awns. In the 1936 crop there occurred among the pure plants a natural cross with short awns and glumes obovate and top nerved and this was the origin of family A.S. 5286. In 1937 this head segregated and gave 138 normal plants, and 54 tiny plants (Fig. 2). The average height of the normal plants was 150 cm., and of the Tiny ones 18 cm. The Tiny plants could be easily spotted in the field in the seedling stage as broad leaved green specks, in marked contrast to the narrow leaved tall-growing seedlings. Only two of these dwarfs produced earheads and the rest withered and died in various stages of growth. No seeds set in the two Tiny plants that flowered.

Later in the same year an  $F_3$  was raised by sowing seed from 10 normal plants. Two of these bred true and 8 segregated and threw Tiny plants. The families segregated for awn length, leaf-sheath colour and glume nerves and shape. The Tiny plants had representatives in all groups of segregates. Being a short duration variety and getting caught up in the rains, many Tiny plants died and what with poor germination, soil wash and disease, no reliable counts could be taken. From one segregating family A.S. 6012, twenty-one further selections were therefore carried forward. It was obvious that the summer season was the best for the pursuit of this character and an  $F_4$  was raised in the summer of 1938. Care was taken to sow the seed in pots keeping a balance of seed for field sowings. Of the 21 selections sown, it is remarkable that 7 came pure and 14 segregated as shown in Table I.

It will be noted that the segregations are monogenic and that the Tiny seedlings are brought about by the loss of a single gene. This gene has been designated  $In_{ty}$ .

Heterozygous earheads were germinated *in situ* and have given the two types of seedlings (Fig. 3) which show out graphically and provide another instance of a graphic method of presenting Mendelian segregations. Similar

TABLE I.

*Seedling segregations.*

Selection Number	Normal	Tiny
A.S. 6012/1	.. Pure	
„ 6012/2		
„ 6012/4		
„ 6012/7		
„ 6012/9		
„ 6012/14		
„ 6012/15		
„ 6012/3	.. 193	60
„ 6012/5	.. 214	61
„ 6012/6	.. 362	118
„ 6012/8	.. 425	140
„ 6012/10	.. 299	103
„ 6012/11	.. 145	46
„ 6012/12	.. 305	95
„ 6012/13	.. 291	98
„ 6012/16	.. 282	102
„ 6012/17	.. 140	44
„ 6012/18	.. 215	67
„ 6012/19	.. 220	75
„ 6012/20	.. 206	58
„ 6012/21	78	29
Total ..	3,375	1,096
Expected ..	3,353	1,118
	P > 0.3	

presentations of Purple-green and Green-albino seedlings have already been published by the senior author (Rangaswami Ayyangar, 1930). The ear-heads are best germinated in wet sand.

Normal and Tiny were measured in detail. Both of them were of the type with steady increase in internodal length (Fig. 4), the number of internodes being on an average 8 in each of them. Internodal and leaf-sheath measurements are given below. They are averages from 10 plants and are from bottom upwards.

TABLE II.

*Length of internodes (cm.).*

Internode No.	1 Bottom	2	3	4	5	6	7	8 Peduncle
Normal ..	0.8	2.1	6.7	11.7	15.5	16.2	19.2	48.9
Tiny ..	0.3	0.5	0.6	0.7	0.8	1.0	1.3	3.6

TABLE III.

*Length of leaf-sheaths (cm.).*

Internode No.	1 Bottom	2	3	4	5	6	7	8 of Flag
Normal ..	Decayed		14.0	15.9	17.4	18.8	20.4	34.2
Tiny ..	3.2	3.6	4.3	5.0	5.6	6.2	7.2	9.3

It will be noticed that the steady increase is kept up in both internodal and leaf-sheath lengths. The remarkable shortening of the internodal length and the comparatively less disparity in leaf-sheath length result in short, thick, stubby plants with a telescopic disposition in the Tiny group. In the case of the normal plants the length of the leaf-blade varied from 30 to 49 cm. whereas in the case of the Tiny it was from 5 to 9 cm. This big contrast in length was not kept up in leaf width. In the normal the leaf width was from 2.6 to 6.1 cm. and in the Tiny from 2.3 to 3.6 cm. It will be seen that with dwarfing there is a big pull down in leaf length; leaf width is comparatively less affected.

The panicles of both normal and Tiny were analysed and the results are tabulated below :

TABLE IV.  
*Analysis of the panicle.*

	Normal	Tiny
Length of panicle, from base to tip .. ..	30.1 cm.	8.0 cm.
Width of panicle (at widest part) .. ..	7.2 ,,	2.8 ,,
Number of whorls .. ..	9	8
Primary branches, number of .. ..	39	24
Do. total length of .. ..	431	65
Secondary branches, number of .. ..	246	80
Do. total length of .. ..	631	89
Number of sessile spikelets .. ..	944	303
Average number of sessile spikelets per secondary branch .. ..	3.8	3.8

It is remarkable that just as the number of internodes is the same in both normal and Tiny plants, the number of whorls on the peduncle is nearly equal. It is also interesting to note that the average number of sessile spikelets per secondary branch is also the same in both.

The panicles differ markedly in their length and width, in the number and length of primary and secondary branches, and in the total number of sessile spikelets. A greater ramification in secondary branches has, therefore, meant a larger number of sessile spikelets.

The spikelets were next examined to find out the cause of the sterility in the Tiny plants. It was seen that the sessile spikelet was nearly of the same size in both the normal and the Tiny plants. So also were the ovary, style and stigma. The difference lay in the anthers. The anthers of the Tiny plant did not dehisce. They were devoid of pollen grains and were very much reduced in size (Fig. 5). Measurements of 100 anthers gave in the normal an average length of 3.5 mm. and an average breadth of 1.0 mm. as against 1.4 mm. and 0.4 mm. in the Tiny. The average lengths of the anther filaments (after anthesis) were 4.0 mm. and 1.8 mm. respectively. Tiny plants selfed in the field failed to set any seed.

An examination of a large number of flowers showed that there could be occasional pollen grains in them. One such instance was noticed in the case of a Tiny plant that set seeds. Of the 104 seedlings raised from this plant 3 proved Tiny and 101 were normal. The 3 Tiny plants grew true to tininess but set no seed, the anthers being completely empty. Of the 101 normal seedlings, 25 were left after thinning and all of them grew to a huge size and gave evidences of being natural crosses. It will thus be seen that whereas the anthers are empty, the stigmas are normal and very receptive to foreign pollen. Artificial crosses were made and in all cases the seeds set.

### Summary.

In sorghum, a new gene designated  $In_{ty}$  is responsible for an extreme reduction in the internodal length, producing Tiny plants. Internodal number is not affected but internodal length only. The wide divergence in internodal length is less graphic in its effect on leaf-sheath length. With reference to the leaf-blade, its length is more affected than its breadth. This gene does not affect the number of whorls on the earhead but has its effect in the panicle having a lesser number and shorter length of secondary panicle branches resulting in fewer spikelets per earhead. Glume size remains practically unaffected. So also ovary and stigma size. The anthers and filaments are reduced in size. The anthers are devoid of pollen. Dwarf plants are therefore usually sterile. Stray pollen grains could be found in some anthers and fertilization by them results in seeds giving true-breeding Tiny plants. The stigmas are very receptive and natural crossing is chronic. Plants heterozygous for the character are the main source of their reproduction. Factor  $in_{ty}$  is a simple monogenic recessive to  $In_{ty}$ .

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## EXPLANATION OF PLATES.

## PLATE XVI.

FIG. 1.—Sorghum varieties differ markedly in height.

*Tall end.*—(1) *Sorghum elegans*—Tanganyika, (2) Ditto, (3) *S. durra*—South India, (4) Ditto, (5) *S. Roxburghii* var. *hians*—S. India, (6) Ditto, (7) *S. subglabrescens*—S. India, (8) Ditto, (9) *S. dochna*—S. India, (10) *S. subglabrescens*—S. India, (11) *S. nervosum*—China, (12) *S. cernuum*—Central India, (13) *S. dochna*—broom-corn—S. Africa, (14) *S. subglabrescens*—milo—S. Africa, (15) *S. caffrorum*—pearl kafir—U.S.A., (16) *S. caudatum*—Chiltex—Texas, U.S.A., (17) *S. caffrorum*—Australia, (18) Ditto, (19) *S. subglabrescens*—beaver milo—Kansas, U.S.A., (20) *S. subglabrescens*—wheatland—Texas, U.S.A., (21) *S. subglabrescens*—two foot milo—U.S.A. *Dwarf end.*

## PLATE XVII.

FIG. 2.—Individual normal and Tiny sorghum plants.

FIG. 3.—A segregating family germinated *in situ* on the earhead, giving tall (normal) and short (Tiny) seedlings.

FIG. 4.—Both normal and Tiny sorghums have the same number of gradually increasing internodes. They differ in internodal length.

FIG. 5.—Normal and Tiny plants differ markedly in anther size.

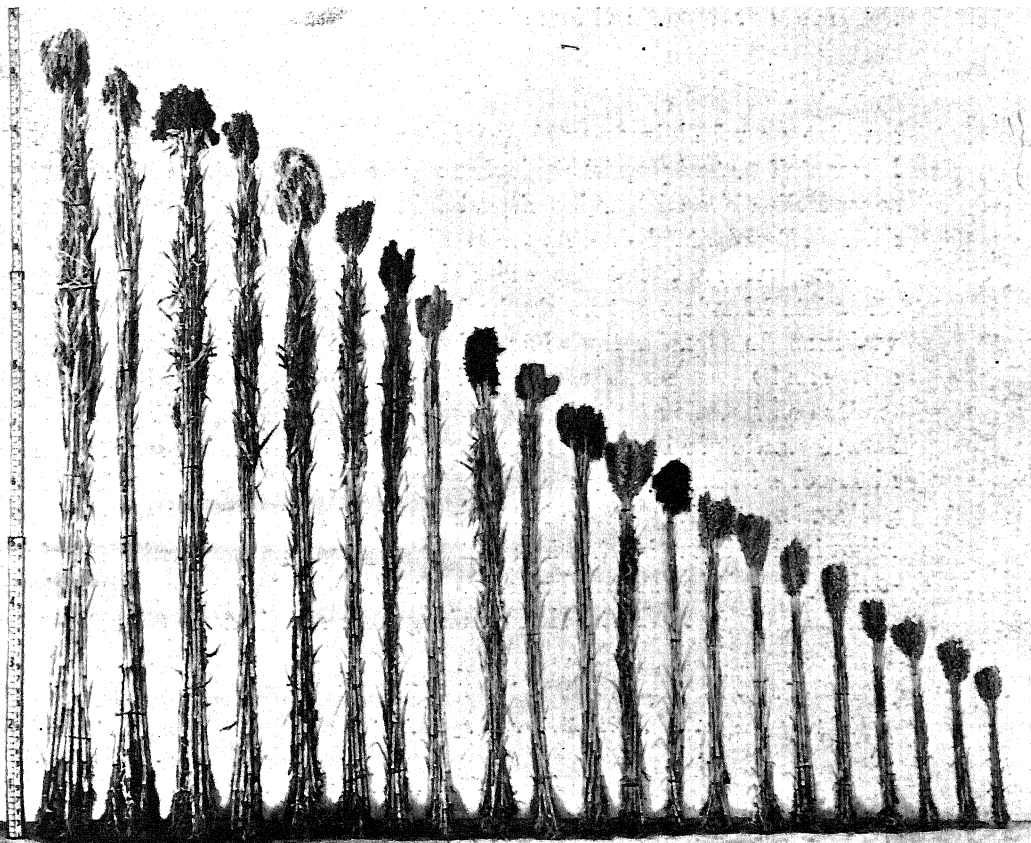


FIG. 1.

FIG. 2.

FIG. 4.

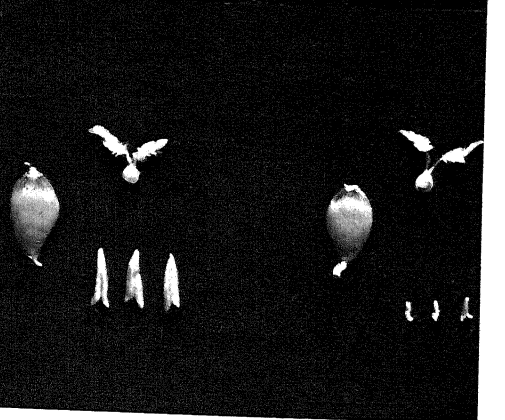
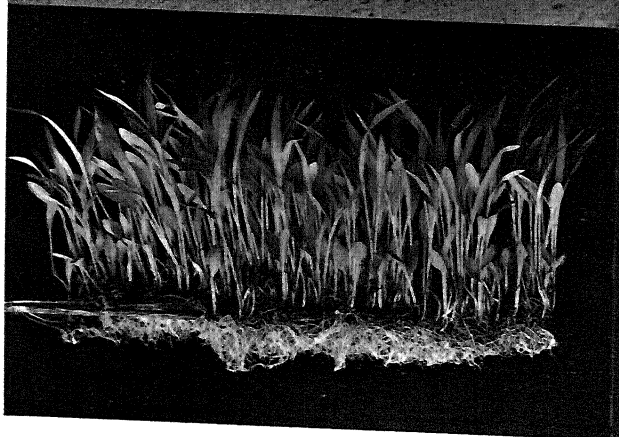
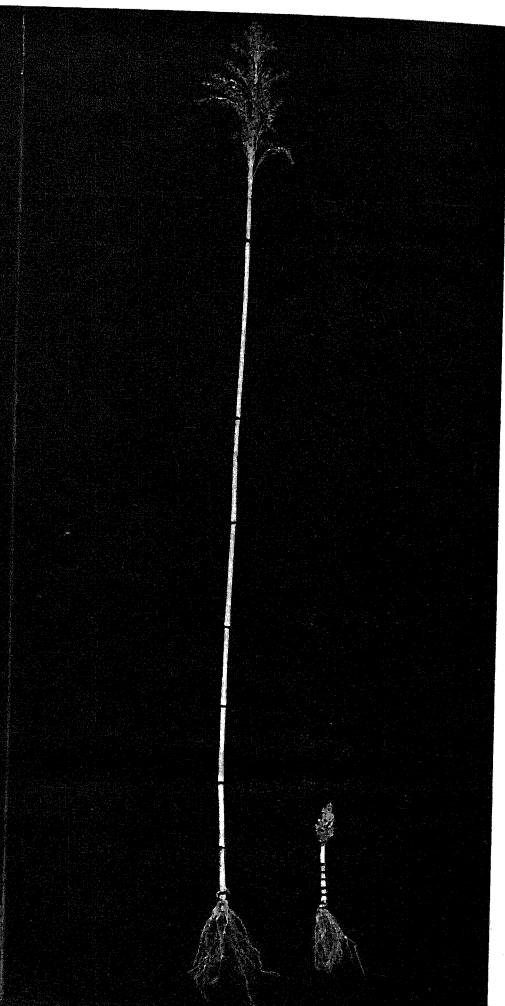
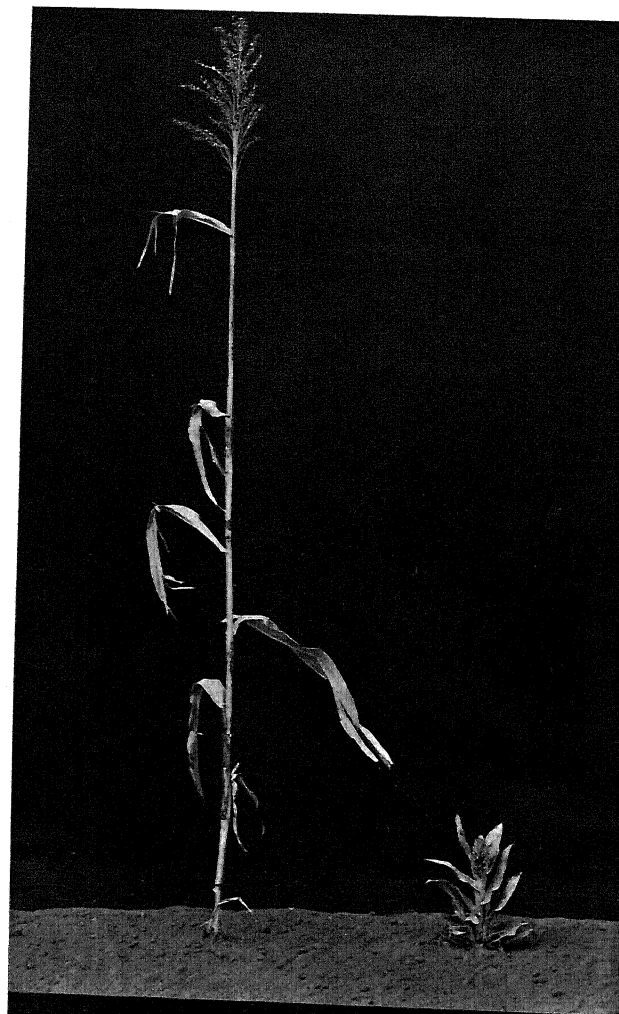


FIG. 3.

FIG. 5.

# THE OCCURRENCE AND INHERITANCE OF PURPLE ANTHERS IN SORGHUM.

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ANTHOCYANIC purple pigment expresses itself in the sorghum plant in various places<sup>1,2,3,4,5</sup> and at various times.<sup>6,7</sup> In almost all cultivated sorghums, the anthers, soon after their emergence from the spikelet, are yellow in colour. The colour of the fresh anthers may be of various shades.<sup>8</sup> When the anthers dry up, they develop different colours which are roughly parallel to the colours that appear on the grain<sup>8,9,10</sup> later on.

Purple pigment in fresh anthers has not been met with in Indian and other Asiatic races of cultivated sorghum. In the wild sorghums, *Sorghum halepense*, belonging to the Eu-Sorghum group and *S. versicolor*, *S. dimidiatum* and *S. purpureo-sericeum* among the Para-Sorghums, the fresh anthers develop in some cases a purple wash close on emergence.

Among the many varieties of African grain sorghums received from Kew, out of the Snowden collection, there were eleven varieties which had purple fresh anthers. Five of them belong to the Caffra, three to the Bicolaria and three to the Guineensia sub-series. These came from Tanganyika, Nyasaland and N. Rhodesia in East Africa and Nigeria in West Africa, and belong to groups *S. caudatum*, *S. coriaceum*, *S. nigricans*, *S. elegans*, *S. Roxburghii* and *S. conspicuum*. Most of them have blackish purple leaf-sheaths and their grain is either brown or has the factor for brown as evidenced by the colour of the dry anther.

In these purple anthered varieties, the anthers emerge coloured purple. The filaments are not so coloured. In the anther, the central groove and the tip and the base of the sacs are more deeply coloured than the rest of the areas. This colour in the fresh anther is in evidence even a day before the spikelets open. On opening, the colour develops quickly in sunlight until the whole anther is coloured well. On cloudy days, the time when the maximum depth of colour is attained is delayed by an hour or two. The

racess with purple anthers vary in the intensity of purple of the fresh anther ; but within their limits of fluctuation they remain constant in manifestation. In anthers at the deeper end of purple, the heterozygous condition in crosses with non-purple anthers, is indicated by a slight pull down in the depth of the purple tint. After the anthers have emerged, opened and shed their pollen they wither and by mid-day they present a violet tint. This tint of violet persists till the anthers become quite dry and by evening the shrivelled anthers lose the violet tint and attain their respective dry anther colours.

When the fresh anthers are coloured purple, the following parts of the sorghum plant are coloured likewise.

1. The plumule, close on emergence from the soil (deeper than similar plumules in non-purple anthered plants).
2. The nodal band—the specialised cushiony tissue at the base of the leaf-sheath that connects the leaf-sheath with the node.
3. The basal portion of the inside of the leaf-sheath (above the inside of the nodal band), for brevity called the Axil. This is coloured a good deep purple.
4. The auricular junction. The outer side is coloured deeper than the inner side. The colour shows in seedlings over 20 days old.
5. The margin of the outer flap of the leaf-sheath. The rudiments of the margin colour are seen even in seedlings 10 days old.
6. The cushiony pulvinar area at the base of the panicle branches, at place of insertion into the main stalk. Ditto, of the branchlets at insertion into the primary branches and the base of the pedicels of the individual spikelets in a faint degree. The pigment develops rapidly at flowering time, keeping pace with the march of flowering.
7. Pedicelled spikelets. These are coloured purple close on emergence, the colour vanishing on drying.
8. Tips of glumes in fertile spikelets.
9. Exposed areas in the roots of the adult plant.
10. The pericarp of the grain at the milky stage.

*N.B.*—In items 2–8 the purple shows best at the flowering stage of the plant.

In the common grain sorghums there is a concurrent manifestation of purple pigment in the following two groups of places:—(1) the plumule *cum* axil, (2) node *cum* junction *cum* leaf-sheath margin. The rarest

occurrence is purple in the anther ; so much so that when this is coloured purple, the whole of the above chain of ten places, become purple.

The first experience in the inheritance of this character was met with in 1934 in Family A.S. 3448, which was a selection from M.S. 1503, a variety from N. Rhodesia, belonging to the group *S. coriaceum*, Snowden. In this family the anthers were noted to segregate, there being 70 plants with purple and 34 without purple in the anthers. The tint of purple varied in the purple group. Six selections were taken, 2 with deep purple anthers and 4 with light purple anthers. The former two bred true and the other four segregated giving a total of 54 deep purple, 101 light purple and 49 no-purple anthered plants. This heterozygous expression of purple in the anther was possible in this family as the mother parent belongs to the deep end of purple in the purple anther varieties. Normally, since the majority of purple anthered plants are not so deep in colour, what could be expected is a simple 3 : 1 ratio of purple anthers to the common yellow anthers, without purple. It is noteworthy that when the segregation occurred, it was not merely for purple in the anther, but for the whole chain of purple in the 10 places detailed above. In this particular family the dilute heterozygous expression was in evidence in all the above places.

The inheritance of this character was pursued through many generations in a family A.S. 3452, N. Rhodesian in origin and belonging to the *S. caudatum* group. A.S. 3452 proved to be a natural cross in the original seed from N. Rhodesia. It had purple anthers in a mother population which had no purple. Thus both the mother and the cross characters are traceable to genes of African origin. In 1934 the  $F_2$  segregation had plants with purple anthers 49 and with no purple 16. Six selections with purple anthers were carried forward. Of these 5 segregated giving a total of 212 plants with purple and 73 with no purple in the anthers. From one of these families, A.S. 4065, thirteen selections were carried forward, 10 purple and 3 yellow anthered. The 3 selections with yellow anthers bred pure. Of the 10 with purple anthers, 4 were pure and 6 segregated again giving a total of 409 purple and 127 yellow anthered plants. A further generation was raised from one of these six families, viz., A.S. 4896, twenty-seven selections being carried forward. The behaviour of the selections is presented in Table I.

More simple monogenic segregations for this character have been met with in 18 other families and their total comes to 541 purple and 183 yellow anthered plants. These include families belonging to *S. caudatum* and *S. Roxburghii* from N. Rhodesia.

TABLE I.

*Behaviour of Selections from Family A.S. 4896.*

Selection Number	Family Number	Character of Selection	Behaviour of Progeny Anthers	
			Purple	Yellow
A.S. 5553	A.S. 4896/1	Purple Anthers	69	32
" 5554	" /2	"	102	30
" 5555	" /3	"	98	33
" 5556	" /4	"	91	26
" 5557	" /5	"	111	31
" 5558	" /6	"	41	15
" 5559	" /7	"	56	17
" 5560	" /8	"	46	15
" 5561	" /9	"	40	13
" 5562	" /10	"	52	22
" 5563	" /11	"	46	14
" 5564	" /12	"	51	22
" 5566	" /14	"	53	19
" 5567	" /15	"	48	17
" 5569	" /17	"	61	21
" 5570	" /18	"	60	18
" 5572	" /20	"	47	20
" 5576	" /24	"	62	21
" 5578	" /26	"	47	24
" 5565	" /13	"	Pure	
" 5568	" /16	"	"	
" 5571	" /19	"	"	
" 5577	" /25	"	"	
" 5573	" /21	Yellow Anthers		Pure
" 5574	" /22	"		"
" 5575	" /23	"		"
" 5579	" /27	"		"
Total ..			1,181	412
Calculated 3 : 1			1,194.75	398.25
$\chi^2 = 0.632$ P > 0.3				

It will thus be seen that a gene designated  $P_{an}$  African in origin (East African and Nigerian) is responsible for the production of purple pigment (anthocyanic) in fresh anthers. Indian and other Asiatic cultivated sorghums lack this gene; a faint trace of purple is however present in the indigenous wild *S. halepense*.

The inter-relationship of this gene  $P_{an}$  with other characters occurring along with it, was gone into and is presented below :

TABLE II.  
*Purple Anther and the B Factor for Pericarp Brown Colour.*

Selection Number	Grain			Brown		White	
	Anther			Purple	Yellow	Purple	Yellow
A.S. 4889	..	..	..	65	27	29	5
„ 4890	..	..	..	48	17	15	7
„ 4894	..	..	..	26	5	7	4
Total			..	139	49	51	16
Calculated 9 : 3 : 3 : 1			..	143.44	47.81	47.81	15.94
$\chi^2 = 0.38 \quad P > 0.9$							

The purple anther behaves independently of one of the B factors determining the brown colour of the pericarp of the grain.

TABLE III.  
*Purple Anther and the Q Factor determining the Colour of the Leaf-sheath.*

Selection Number	Leaf-sheath			Reddish-Purple		Blackish-Purple	
	Anther			Purple	Yellow	Purple	Yellow
A.S. 4889	..	..	..	73	26	21	6
„ 4890	..	..	..	51	17	12	7
„ 4894	..	..	..	24	6	8	4
„ 4899	..	..	..	55	19	20	3
Total			..	203	68	61	20
Calculated 9 : 3 : 3 : 1			..	198	66	66	22
				$\chi^2 = 0.75 \text{ P} > 0.8$			

Factor  $P_{an}$  is independent of the Q factor.

TABLE IV.

*Purple Anther and Brown Colour in the Nucellus.*

Selection Number	Anthers			Purple		Yellow	
	Nucellar layer			Brown	No Brown	Brown	No Brown
A.S. 4896	..	..	..	57	13	14	4
„ 4906	..	..	..	36	9	8	7
„ 5553	..	..	..	46	23	27	5
„ 5554	..	..	..	78	24	24	6
„ 5557	..	..	..	83	23	28	8
„ 5558	..	..	..	32	9	11	4
Total				332	101	112	34
Calculated 9 : 3 : 3 : 1				326	108.5	108.5	36
					$\chi^2 = 0.85$ $P > 0.8$		

$P_{an}$  is independent of the factor determining the expression of brown colour in the nucellar layer of the grain.

The above experiences are from families purely African in origin. Crosses between purple anthered African races and yellow anthered Indian races are under examination and the results of this impact between these divergent races will be published in due course.

#### Summary.

Anthocyanic purple pigment expresses itself in the sorghum plant in various places and at various times. This occurrence could be grouped into specific chains of manifestation. The anther is rarely coloured purple. When it is so coloured, it is one among the following ten places in its chain : plumule, nodal band, axil, auricular junction, leaf-sheath margin, pulvinus of the panicle branches, pedicelled spikelets, glume tips, exposed roots and pericarp of the grain at milky stage. The expression of this purple in freshly emerging anthers is noted in some races of cultivated sorghum, East African and Nigerian in origin, belonging to the groups *S. caudatum*, *S. coriaceum*,

*S. nigricans*, *S. elegans*, *S. Roxburghii*, and *S. conspicuum*. Purple in the anthers occurs in the wild sorghums *S. halepense* of the Eu-Sorghum group, and *S. versicolor*, *S. dimidiatum* and *S. purpureo-sericeum* of the Para-Sorghum group. A gene designated  $P_{an}$  is responsible for this expression of purple in the anther. In its absence ( $p_{an}$ ) the anther is of the ordinary yellow colour.  $P_{an}$  is a simple dominant to  $p_{an}$  in crosses between African races. Factor  $P_{an}$  behaves in inheritance independently of one of the B factors determining the brown colour of the grain of the Q factor conditioning the colour of the leaf-sheath and of the factor responsible for the production of brown colour in the nucellar layer of the grain. The occurrence of this dominant gene along with other similar dominant pigment genes<sup>6,7</sup> in African races and their disappearance in the Indian and other Asiatic races throws light on the origin and evolution of cultivated sorghums.

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# THE EFFECT OF CHLORINE IN RELATION TO AGE UPON THE GROWTH AND COMPOSITION OF WHEAT

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## *Introduction*

IN a previous publication<sup>16</sup> it was pointed out that among the so-called non-essential elements absorbed by plants there are some which, in small doses stimulate plant growth. Of these, chlorine is one, investigations regarding the nutritive value of which have led to conflicting views. While one school of workers<sup>8,10,11</sup> has stressed the importance of chlorine as an essential element in plant growth, the other<sup>4,5,20,21,22</sup> holds the view that aside from the little amount of chlorine contained in the seed, plants do not need this element for their normal growth and development.

A review of literature on this subject has already found prominence in Tottingham's work<sup>18,19,20</sup> which reveals that the response of plants to chlorine depends upon the amount of this element supplied, the species of plant investigated and the environmental conditions prevailing during experimentation. A lack of realisation of the response made by plant to chlorine relative to its age and developmental stage as a vital determinant, however, calls to importance a more detailed study of the problem in the light of improved methods and experimental technique. The present investigation deals with this aspect of the problem in which the influence of this element has been studied at successive growth stages of wheat growing in Knop's solution under controlled conditions, when this element has been supplied in a suitable concentration for the entire as well as parts of the life-cycle. The age-accruing morphological and compositional changes have been traced in their details and finally discussed with special reference to the physiological rôle of this element relative to the age and developmental stage of the plant.

## *Investigation*

Sterilised seeds of wheat (var. Pusa 4) of as uniform a size and relative density as possible were germinated on mosquito nettings tied to the edge of germination dishes and moistened with those culture solutions in which the plants were subsequently grown. Seedlings of practically equal size and vigour were afterwards transferred to culture jars of hard resistant

glass, fifteen litres in capacity. The following six sets of cultures were used for experimentation :—

- (a) Plants raised throughout in Knop's solution, designated as the *control medium*.
- (b) Plants grown in Knop's solution containing 0.005 M potassium chloride\* termed as the *chloride medium*.
- (c & d) Plants initially raised in Knop's solution but later on transferred to chloride medium after twenty and thirty-five days—*sets III and IV* respectively.
- (e & f) Plants initially grown in chloride medium but afterwards transferred to Knop's solution after similar intervals of twenty and thirty-five days—*sets V and VI* respectively.

The culture solutions in all the six sets were renewed at intervals of three days and aerated daily by means of a compressor connected to a reservoir. Twenty-four plants were grown in each jar in the water culture room where the different sets grew under controlled conditions of light† (intensity 80,000 m.c.). To avoid the heating effect of the rays upon the plants, a 3-inch screen of running water was interposed between the source of illumination and the plants. The temperature above the plants, thus, did not fluctuate beyond  $\pm 0.9^{\circ}\text{C}$ . while the culture jars containing the entire roots being inside a thermostatically controlled bath, the absorption of chlorine took place at a temperature of  $25.0^{\circ}\text{C}$ . The air inside the room was kept in constant circulation by means of electric and suction fans which refreshed the surrounding air of the plant and helped in controlling the humidity of the room within  $\pm 4\%$ . The plants grew under atmospheric concentrations of  $\text{CO}_2$  (0.040–0.045%) and  $\text{O}_2$  (20.98%).

Thirty to fifty plants belonging to each of the treated and the control sets were taken out of the culture solution every week and the shoot and root lengths, assimilating leaf surface and fresh weight recorded. The plants were then dried in a steam oven to constant weight. The carbohydrate fractions were estimated after Lane and Eynon<sup>7</sup> the total nitrogen by the Kjeldahl's method modified to include the nitrate nitrogen.<sup>17</sup> In all such cases the material for analysis was prepared according to the method suggested by Link and Tottingham.<sup>9</sup> A duplicate analysis of each sample was made and the

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\* This concentration of potassium chloride as against higher or lower concentrations was found to be most suitable for the growth of wheat plants by previous preliminary experimentation (see Fig. 5).

† This light intensity was found to be above the limiting value in the previous experimentation (cf. Singh, B. N., and Kumar, K., 1935).

average of the two was finally recorded. The diastase was prepared and its activity measured by the rate of sugar formation in a starch digest incubated at 38° C. at pH 5.2 (*cp.* Loomis and Shull<sup>12</sup>). The diastatic activity was found both in presence and absence of potassium chloride in 0.005 M concentration.

### Experimental Results

*Dry matter production*.—The dry matter accumulation in plants belonging to all the six sets, in spite of slight variations noted during successive intervals of the age-cycle, shows a progressive increase from week to week (Fig. 1).

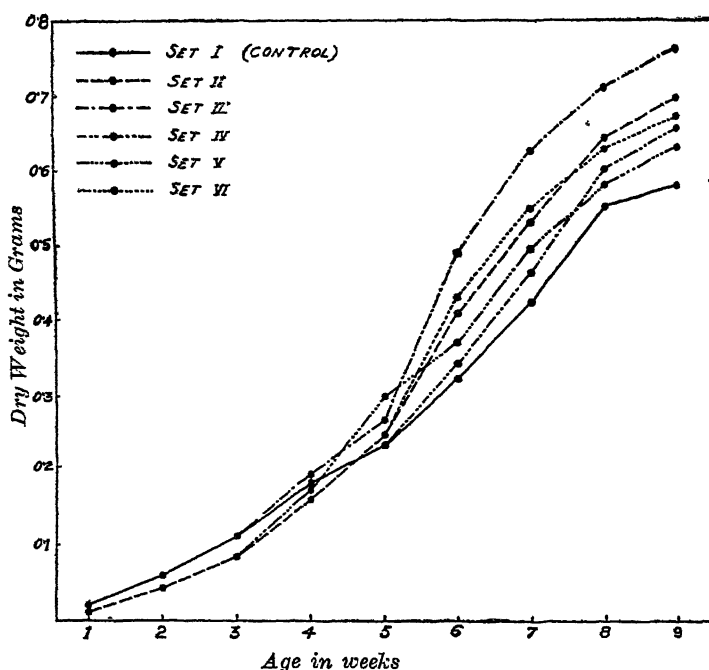
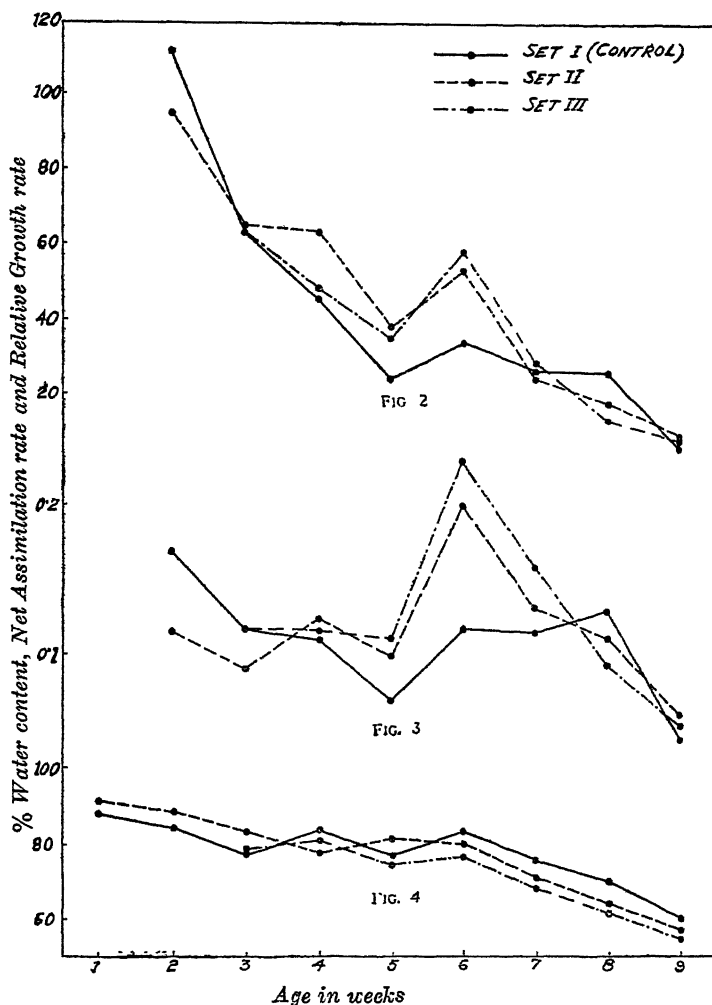


FIG. 1. Time-weight curves per plant belonging to different sets

Soon after their transference into the culture media plants of set II to which chlorine was supplied from the very beginning of the life-cycle, seem to have a retarded growth as testified by their general appearance and lower growth rate (Fig. 2) as compared to the control ones (set I). Towards later periods of the life-cycle, however, when the plants have attained an age of twenty-eight days, the growth is more accelerated in case of set II as revealed by a higher growth rate obtained over the control (Fig. 2). The final dry matter yield in the treated plants is, consequently, increased by 18.49%. It appears, therefore, that an extra supply of chlorine beyond that contained in the seed may have a retardatory influence on plant growth during the

early stages, while at a later period, when the available amount present in the seed is possibly used up, does an additional supply of this element stimulate dry matter accumulation. Plants belonging to set III, however, where



FIGS. 2, 3 and 4 represent relative growth rate, net assimilation rate and water content per plant respectively

chlorine is added twenty days after germination, maximum dry water formation is obtained, an increase of 30.84% in final dry matter yield being calculated over the control. In the remaining treated sets, viz., sets VI, IV and V, the final dry matter production although decreasing in descending order maintains a higher level than that of the control.

TABLE I  
*Morphological characters in wheat as influenced by chlorine administered at different stages of growth*

Set No.	Treatment	Particulars per plant					
		Final dry weight in grams			Shoot length cm.	Leaf area Sq. cm.	Increase in ear formation %
		Root	Shoot	Entire plant			
I	Control (Knop's solution) ..	0.123 ± 0.003	0.464 ± 0.012	0.587 ± 0.019	24.7 ± 0.721	85.2 ± 1.787	..
II	Chloride solution ..	0.145 ± 0.005	0.550 ± 0.019	0.695 ± 0.027	20.8 ± 0.497	91.4 ± 1.876	13.79
III	Plants transferred from Knop's to chloride solution after 20 days ..	0.180 ± 0.006	0.588 ± 0.017	0.768 ± 0.029	24.1 ± 0.806	96.7 ± 2.201	27.71
IV	Plants transferred from Knop's to chloride solution after 35 days ..	0.141 ± 0.005	0.515 ± 0.015	0.656 ± 0.026	23.9 ± 0.937	93.1 ± 2.162	15.21
V	Plants transferred from chloride to Knop's solution after 20 days ..	0.132 ± 0.003	0.504 ± 0.014	0.636 ± 0.023	23.5 ± 0.485	90.1 ± 2.105	16.43
VI	Plants transferred from chloride to Knop's solution after 35 days ..	0.141 ± 0.004	0.535 ± 0.015	0.676 ± 0.021	22.8 ± 0.856	92.7 ± 1.985	18.33

*Shoot and root length*.:—Accompanied with such characteristic variations in the dry matter production, the shoot and root lengths also exhibit wide differences at different life-stages from set to set (Table I). The final root length in the treated (set II) and the control plants (set I) is  $20.8 \pm 0.497$  and  $24.7 \pm 0.721$  cms. respectively. The treated plants, however, in spite of this diminution in length exhibit greater side way ramification which evidently leads to increased dry matter production in their roots as compared to the control ones.

*Leaf area*.:—The values obtained for the assimilating surface in case of sets II and III show a decided increase over the control (Table I). In the other sets, however, no such marked variations are observed.

*Water content*.:—The plants belonging to the different sets show slight fluctuations with regard to their water content during their early stages of growth (Fig. 4), whereas with the approach of maturity there follows a rapid decrease. The treated plants (set II), however, have a higher moisture content than the control ones in earlier stages while a decrease is obtained later in this direction.

*Ear formation*.:—The percentage ear formation in cases where chlorine is supplied either throughout or only for parts of the life-cycle, is increased over the control plants (Table I). The highest percentage is, however, obtained in plants belonging to set III, while in the remaining sets the variations are not so significant.

*Carbohydrate fractions and nitrogen content*.:—The reducing sugars in the treated plants (sets II and III) are lower as compared to the control plants during the early as well as ear formation stages, whereas a slow increase is to be obtained after the flowering period (Table II). No marked increase over the control is observed in the remaining treated sets. The sucrose content as well is reduced below the control during early stages, while reverse is to be noted towards the close of the life-cycle. The starch content, on the other hand, is higher in the treated plants (set II) during the early periods, but after fertilization when it exhibits a sudden rise in both the treated and the control sets the level of increase over the control is not so marked. The remaining treated sets do not show any great difference in their final starch content.

An inter-comprison among the various carbohydrate fractions reveals that while the accumulation of different sugars in the treated and the control sets exhibits similar march with age, differences are obvious in their relative concentrations. Thus, as the reducing sugars reach the highest limit when the plants are about four weeks old, the sucrose percentage attains the lowest values; while near about the flowering period when the reducing sugars are

TABLE II  
*Content of carbohydrate fractions, total nitrogen and C/N ratio at successive stages of growth of wheat*

Days	Percentage dry weight basis					
	Reducing sugars	Sucrose	Starch	Total carbo-hydrates	Total nitrogen	C/N
<i>Set I (Control)</i>						
8	5.45	9.07	9.82	23.89	5.73	4.17
15	8.25	8.48	8.15	23.88	4.65	5.12
26	20.05	6.60	7.21	33.86	3.95	8.37
33	21.29	5.13	6.47	32.89	3.73	8.65
49	10.03	6.56	5.99	21.58	2.91	8.98
56	13.07	8.37	13.90	35.34	3.58	9.88
63	12.12	9.41	19.72	41.25	3.77	10.94
<i>Set II (Chloride solution)</i>						
8	4.82	7.20	16.71	28.73	5.82	4.93
15	6.28	6.52	17.32	30.12	4.97	6.06
26	18.09	5.07	17.78	40.94	3.78	10.83
33	15.73	11.20	16.34	43.27	3.56	11.57
49	10.78	11.10	13.27	35.15	2.93	11.99
56	15.73	13.50	15.82	43.03	3.96	10.73
63	15.27	11.85	18.57	45.69	4.12	10.95
<i>Set III (Plants transferred from Knop's to chloride solution after 20 days)</i>						
26	17.83	7.12	7.27	32.22	3.85	8.37
33	18.08	6.97	6.73	31.78	3.71	8.56
49	11.12	11.28	6.08	28.48	2.83	10.06
56	15.60	10.25	19.29	45.14	3.61	12.50
63	16.53	10.13	19.08	45.74	3.79	12.07
<i>Set IV (Plants transferred from Knop's to chloride solution after 35 days)</i>						
49	12.68	9.38	7.25	29.31	2.78	10.54
56	16.23	7.78	17.85	41.86	3.51	11.92
63	14.98	7.52	19.98	41.48	3.87	10.67

TABLE II—(Contd.)

Days	Percentage dry weight basis					
	Reducing sugars	Sucrose	Starch	Total carbohydrates	Total nitrogen	C/N
<i>Set V (Plants transferred from chloride to Knop's solution after 20 days)</i>						
26	18.00	7.05	16.11	41.16	3.89	10.55
33	16.17	9.97	15.93	42.07	3.79	11.12
49	9.42	8.23	10.98	28.63	2.53	11.23
56	16.21	9.76	18.50	44.47	3.79	11.73
63	14.21	8.25	20.93	43.39	3.64	11.92
<i>Set VI (Plants transferred from chloride to Knop's solution after 35 days)</i>						
49	12.10	11.80	10.97	34.87	3.15	11.07
56	16.31	9.68	18.80	44.79	4.81	9.31
63	14.37	9.41	21.12	43.90	4.63	9.63

at the lowest, the sucrose increases. After fertilization, the starch content also exhibits a sudden rise accompanied by a slow increase of the reducing and a decrease of the non-reducing sugars. Such characteristic variations in the relative concentrations of sucrose and starch are partially correlated with the high or low water content of the treated plants (set II) during their early as well as final stages of growth (*cf.* Lundegardh, 1914 and Bruns, 1925).

The total carbohydrates in the different sets exhibit a marked increase at two distinct stages (Table II), one when the plants are approximately thirty days old (pre-flowering stage), and the other when they are about sixty days (seed formation stage). Chlorine-treated plants, in general, have greater percentage of total carbohydrates than the check ones.

The nitrogen content exhibits a decline till the seventh week prior to the seed formation stage after which a rise is observed (Table II). The treated plants, however, do not show any marked increase in this direction (*cf.* Breazeale, 1916).

The C/N ratio in both the treated and control sets rises from the very outset reaching a maximum prior to blossoming after which it declines. The treated sets in general indicate a higher ratio at successive stages of growth (Table II). This shows that increased growth is invariably associated with increased C/N ratio.

*Net assimilation rate* :—The values obtained for the assimilation rate also undergo marked changes like those obtained for the carbohydrates (Fig. 3). Plants of set III have the highest net assimilation rate followed by those of sets II and I. A marked correlation between the assimilation and growth rate curves as also the carbohydrate accumulation in different sets is observed.

#### Discussion

When the dry weight of plants belonging to different sets is plotted against age (Fig. 1), a sigmoid curve is obtained. At any stage in the life-cycle an increase in dry matter accumulation takes place by either an increase in age or the factor for chlorine, the optimum effect being noticed under the concentration of 0.005 M potassium chloride (Fig. 5). It appears,

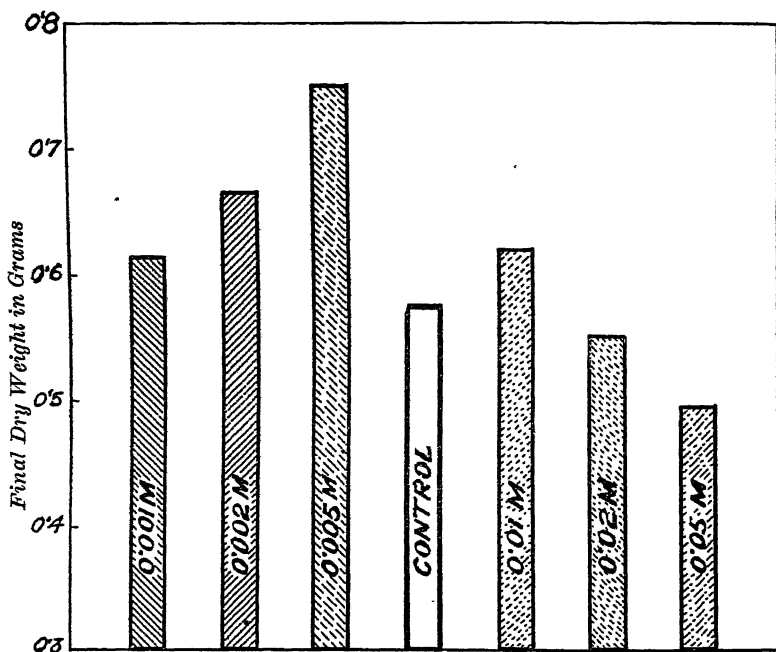


FIG. 5. The final dry matter yield as influenced by different concentrations of potassium chloride

therefore, that growth of plants in the present instance is the resultant of the conjoint influence of both the factors for chlorine as well as the age of plants.

It is further noted from the foregoing observations that when chlorine in 0.005 M concentration is supplied to the plants from the very beginning of their life-cycle there is little beneficial effect produced during the early stages. This is testified by the diminished dry matter yield and a lower

growth rate obtained in the treated plants (set II) as compared to the control ones (Set I), within this period. But if this element is added at the stage when the plants are showing active growth and development highest dry matter yield is obtained, as observed in case of set III where the plants exhibit maximum growth with largest percentage ear formation and have the highest net assimilation and relative growth rates (Figs. 3 and 2). These facts lead us to the view that the greatest physiological need and hence maximum stimulative efficiency of this element is felt during the juvenile stage when its presence is found to bring about useful after-effect.

The manner in which this element brings about such characteristic responses in plants, has been variously suggested by different investigators. Rudolfs<sup>14</sup> has pointed out the effect to be due to the increased or decreased acidity in the cell sap which in its turn stimulates or retards the enzymatic activity of plants. Kellerman<sup>6</sup> and Hawkins<sup>3</sup> as well as Tottingham<sup>20</sup> have opined that chlorine in plants influences the various intra-cellular enzymes especially the diastatic ones. For this purpose, the effect of potassium chloride on the activity of diastase complex extracted from leaves of the plants belonging to different sets was found. A glance on the activation ratio:  $\frac{\text{Activity in presence of potassium chloride}}{\text{Activity in absence of potassium chloride}}$  (Table III) reveals that there is a greater diastatic activity observed in presence of potassium

TABLE III  
*The influence of chlorine on the diastatic activity of wheat*

Set No.	Treatment	Diastatic activity in terms of reducing sugars formed by 1 gm. of dry material in one hour		
		With KCl added to digest mgms.	Without KCl added to digest mgms.	Activation ratio
I	Control (Knop's solution) .. ..	275	201	1.37
II	Chloride solution .. ..	319	225	1.42
III	Plants transferred from Knop's to chloride solution after 20 days .. ..	347	227	1.53
IV	Plants transferred from Knop's to chloride solution after 35 days .. ..	293	211	1.39
V	Plants transferred from chloride to Knop's solution after 20 days .. ..	296	207	1.43
VI	Plants transferred from chloride to Knop's solution after 35 days .. ..	289	213	1.35

chloride in all the cases and that the ratio is higher in most of the treated sets. This indicates that there is good probability of such an activation of diastase complex occurring in plants, in presence of potassium chloride in the plant sap. The increased efficiency in carbohydrate synthesis, net assimilation and relative growth rates in the treated plants (sets II and III), when considered along with the increased leaf surface suggests that acceleration in growth of the treated plants may as well be related to the augmentative influence of chlorine on photosynthetic reactions.

### *Summary*

The application of chlorine from the very beginning of the life-cycle of wheat, brings about a slight depression in dry matter production and an increase in moisture content during their early stages of growth. With advance in age, however, the dry matter accumulation is markedly increased without any appreciable increase in water content.

Plants supplied with chlorine twenty days after germination have the maximum dry matter yield. Relative growth rate as well as net assimilation rate are also accelerated.

The treated plants do not show any marked effect on their shoot length although the assimilatory surface is increased beyond the control. The roots, on the other hand, in spite of a diminution in length, exhibit a greater lateral ramification and dry matter production.

Chlorine-treated plants have a larger percentage ear formation maximum values being obtained in case where this element is supplied twenty days after germination.

The accumulation of carbohydrates as also the diastatic activity are greater in the treated plants as compared to the control ones.

Growth of plants at successive stages of the life-cycle appears to be determined by both the age and the factor for chlorine a change in either bringing about a variation in dry matter production.

The greatest physiological need and hence the maximum augmentative efficiency of this element is found at the period (twenty days after germination in the present case) when plants are showing active growth and differentiation.

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# OBSERVATIONS ON SOME ZYGNEMALES FROM NORTHERN INDIA—PART II.

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*Spirogyra* Link (1820).

FILAMENTOUS habit, rarely branched. Septa plain, semireplicate, replicate, or colligate. Each cell with one or more spiral chromatophores, each bearing numerous pyrenoids. Sexual reproduction by zygospores, conjugation scalariform or lateral. Asexual reproduction by aplanospores or akinetes. Zygospore-wall composed of 2–3 layers of which the mesospore may be smooth or sculptured and yellow to yellowish brown when mature.

## 1. *Spirogyra aplanosporum* sp. nov.

(Fig. 31.)

Vegetative cells 20–26  $\mu$  broad, and 2–4 times, as long. Septa plain. Each cell contains a single spiral chloroplast with 3–6 spirals (Fig. A).

*Reproduction.*—Reproduction in this species takes place exclusively by means of aplanospores. In most filaments the fertile cells become swollen on both sides, appearing almost globose in shape (Fig. B), while in some filaments these become swollen on one side only (Figs. E, F). Abortive conjugation tubes are given out by some of the cells, which may be closed or ruptured, presenting the appearance of a broken conjugation canal. In one peculiar case the tube had become swollen in a balloon-like manner and an aplanospore was found lodged in it (Fig. E). The aplanospores are globose to ellipsoid in shape, and are 24–30  $\mu$  broad and 30–54  $\mu$  long. The spore-wall is composed of two layers, a light-blue exospore and a brown mesospore, both of which are smooth. In most cases the sterile cells become swollen in a globose fashion and the chloroplast is in the shape of a ring or a loop (Figs. C and D). In some cases even the sterile cells give out conjugation-canal-like protuberances, on one or both sides (Figs. F and G). The sterile swollen cells may be as broad as 54  $\mu$  in some cases.

*Affinities.*—The nearest related species is a Chinese one, *S. subpapulata* Jao, from which the Indian form differs in the entire absence of scalariform conjugation, its smooth spores, and its rounded sterile swollen cells.

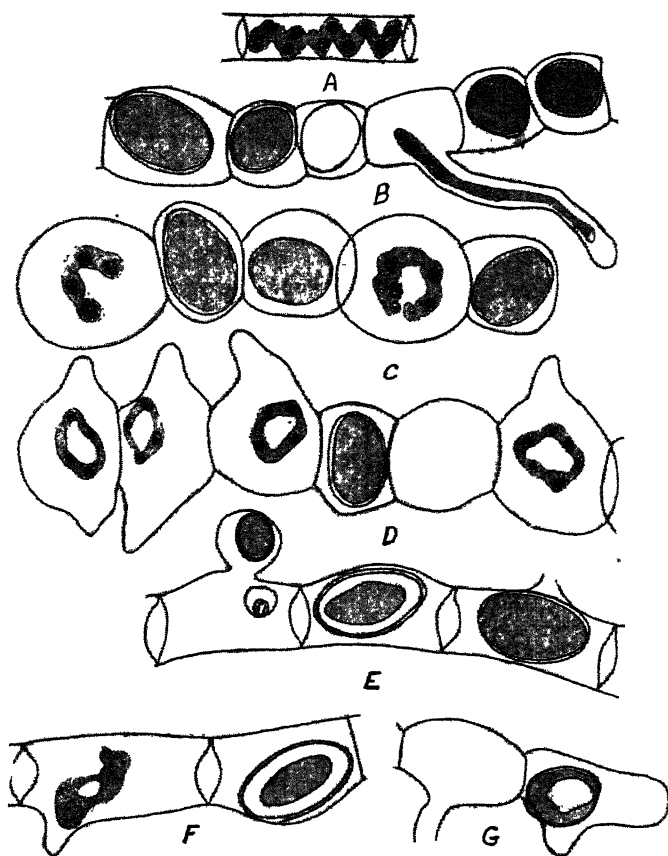


FIG. 31. *Spirogyra aplanosporum* sp. nov.

A.—Shows a vegetative cell.

B.—Shows aplanospores in a filament.

C. and D.—Show swollen sterile cells and aplanospores.

E.—Shows an aplanospore formed in a swollen conjugation canal-like structure.

F. and G.—Show abortive conjugation canals given out by some of the cells.

× 310.

*Habit.*—Collected from a pond near Dasuya, Punjab, in January 1929.

## 2. *Spirogyra flavescens* (Hass.) Kutz.

*Op. cit.*, Czurda, *Susswasserflora Mitteleuropas*.

Vegetative cells 12–14  $\mu$  broad, and many times as long. Septa plain. There is only one very thin, more or less straight chloroplast in each cell.

*Conjugation*.—Both scalariform and lateral conjugation are seen in this species. The female cells are distinctly swollen. The zygospores are long, ellipsoid in shape, are  $18-20\ \mu$  broad, and  $40-50\ \mu$  long. The zygospore wall is composed of two layers only, a thin brownish exospore, and a bluish green mesospore. The surface of the zygospore bears reticulate markings and depressions.

So far as the author is aware this is the first description of the zygospores of this species. The description of this species as given by Czurdæ does not give any figure, and no details about zygospore-formation are given.

*Habit*.—Found free-floating along with *Zygnema Czurdæ* and a species of *Oedogonium* during the third week of February 1931, in a fresh-water spring at Tahli Sahib, district Hoshiarpore, Punjab.

### 3. *Spirogyra Skujæ* sp. nov.

(Fig. 32.)

Vegetative cells  $14-17\ \mu$  broad, 6–10 times as long, each with a single spiral chloroplast of 3–5 turns. Septa plain and swollen (Fig. A).

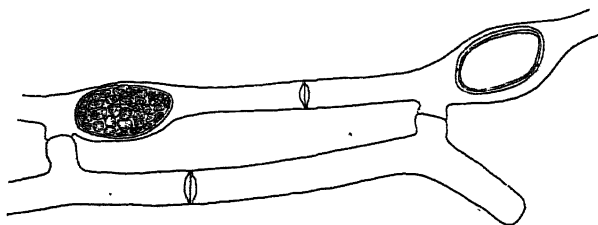


FIG. 32. *Spirogyra Skujæ* sp. nov.

× 310.

*Reproduction*.—Conjugation scalariform. Fruiting cell swollen on both sides  $26-34\ \mu$  in diameter. Zygospores oval, yellowish in colour,  $24-30\ \mu$  broad and  $40-42\ \mu$  long. Zygospore-wall composed of two layers, a thin and smooth exospore, and yellowish mesospore with reticulations and wavy lines on surface (Fig. B).

*Affinities*.—In the size of its cells and zygospores this species resembles *S. fennica* Cedercreutz but differs from it in having reticulations and wavy lines on its spore-wall.

*Habit*.—Found free-floating mixed with *Zygnemopsis lamellata* and *Oedogonium* sp. in a Jhil near Tanda, district Fyzabad, U.P., on 7th February 1937.

4. *Spirogyra reticuliana* sp. nov.

(Fig. 33.)

Vegetative cells 14–16  $\mu$  broad, 10–14 times as long, with plain end walls. One chloroplast with 2–4 spirals (Fig. A), and in some cases more or less straight.

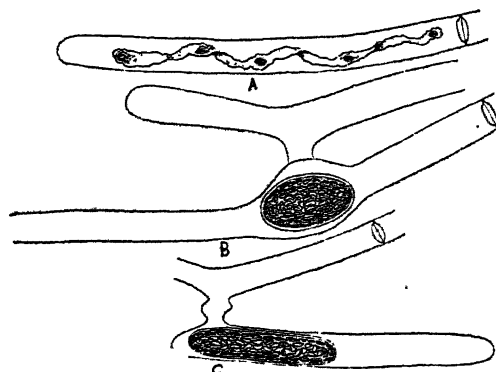


FIG. 33. *Spirogyra reticuliana* sp. nov.

A.—Shows a vegetative filament with chloroplasts.

B.—Shows conjugation with ripe zygospores.

C.—Shows an abnormal zygospore.

× 310.

*Conjugation*.—Conjugation scalariform. Conjugation tubes are formed by the male gametangia only. Zygospores 23–26  $\mu$  broad, 40–45  $\mu$  long spore wall yellow, composed of a thin light-bluish exospore, and a thicker brownish mesospore, with foveolate reticulations (Fig. B). Some elongated cylindrical abnormal zygospores were also seen in some cells (Fig. C).

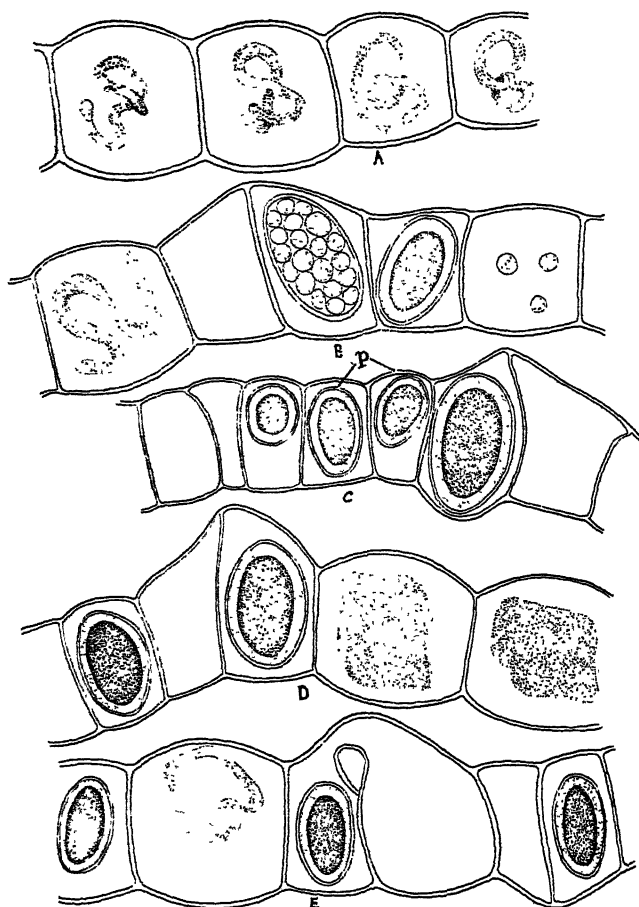
*Affinities*.—In the dimensions of its vegetative cells and zygospores it resembles *S. liana* Transeau, but differs from it in having reticulations on the surface of spore-wall more or less straight nature of its chloroplasts and the absence of sterile cells separating the conjugating cells. These differences are sufficient to warrant the establishment of a new species which is named as *S. reticuliana*.

*Habit*.—Collected from a stagnant drainage channel near V. Mamrezipur tehsil Tanda, district Fyzabad, in the middle of January 1937.

5. *Spirogyra Sahnii* sp. nov.

(Fig. 34.)

This alga was found mixed with filaments of *Sphaeroplea annulina*, free floating in Siah Baeen, a fresh-water stream near Dasuya, Punjab, about the middle of March 1931.

FIG. 34. *Spirogyra Sahnii* sp. nov.

- A.—Shows a vegetative filament, with cells containing coiled chloroplasts.  
 B.—Shows lateral conjugation. Mark the fungus spores inside a zygospore.  
 C.—Shows a zygospore and 2 parthenospores (p).  
 D.—Shows a filament showing lateral conjugation.  
 E.—Shows a peculiar method of conjugation with the help of a tubular structure.

All  $\times 310$ .

Vegetative cells are  $48-72\ \mu$  broad and  $40-74\ \mu$  long. Usually they are broader than long. They are very much swollen and are barrel-like in appearance. There is a single chloroplast which is more or less coiled in an irregular fashion. The septa of the cells are plain (Fig. A).

*Reproduction.*—Only lateral conjugation has been noticed in this alga, and this is of a very interesting type. The neighbouring cells usually give

out tent-like protuberances in the usual way, and the female cells containing the zygospores almost always adjoin empty male cells (Figs. B and D). The female cells are usually of the same size, but in one case the empty male cell was considerably swollen and much bigger in size. It gave out a distinct tube which was continuous with a similar structure given out by the female cell, and appeared like a retort (Fig. E). Such conjugation tubes have been reported by de Bary in *Zygnema insigne* (Hassal) Kutz.

*Parthenospores*.—Parthenospores are also seen in large numbers along with the zygospores. These are usually oval in shape like the zygospores, but are very much smaller in size, being 20–24  $\mu$  broad, and 22–36  $\mu$  long. In some cases these are spherical in shape (Fig. C).

Some of the cells are infested with a fungal parasite, similar to a species of *Myzocitium* described on a material of *Spirogyra affinis* by Choudhari. Some of the zygospores also are full of the cells of this parasite (Fig. B). It is a curious coincident that both the species of *Spirogyra* in which this form of *Myzocitium* has been seen reproduce by lateral conjugation.

Zygospores are 22–36  $\mu$  broad, and 44–68  $\mu$  long. The zygospore-wall is composed of three layers, a smooth hyaline exospore, a thick bluish-green mesospore, and a smooth endospore.

In one filament, the cells were noticed to produce conical protuberances, which give them a pear-shaped appearance. Probably these are abortive conjugation canals.

*Affinities*.—There are four species of *Spirogyra* which resemble the present form in some features, and specially in the possession of a single chloroplast and lateral conjugation. Of these it differs from *S. longata* (Vauch.) Czurda and *S. Lagerheimii* Wittrock in the size and shape of vegetative cells and zygospores. From *S. condensata* (Vauch.) Czurda emend, it differs in the shape and size of vegetative cells, the size of zygospores, and in the presence of parthenospores. The fourth species is *S. asiatica* Czurda from which this alga differs in the shape of vegetative cells, presence of parthenospores, and the absence of any punctation from the mesospore as well as its bluish-green colour.

I have named this species of *Spirogyra* after Dr. Birbal Sahni of Lucknow University, who has done so much to raise the prestige of Indian Botany.

*Habit*.—Free-floating in Siah Baeen, a fresh-water stream, mixed with *Sphæroplea annulina*, near Dasuya, district Hoshiarpore, Punjab, about the second week of March 1931.

6. *Spirogyra parvula* (Trans.) Czurda nov. comb.  
*Op. cit.*, Czurda, *Susswasserflora Mitteleuropas*, Heft 9.

(Fig. 35.)

Vegetative cells 20–24  $\mu$  broad, 2–5 times as long. Each cell with one chromatophore of 2–4 turns. Septa plain (Fig. A).

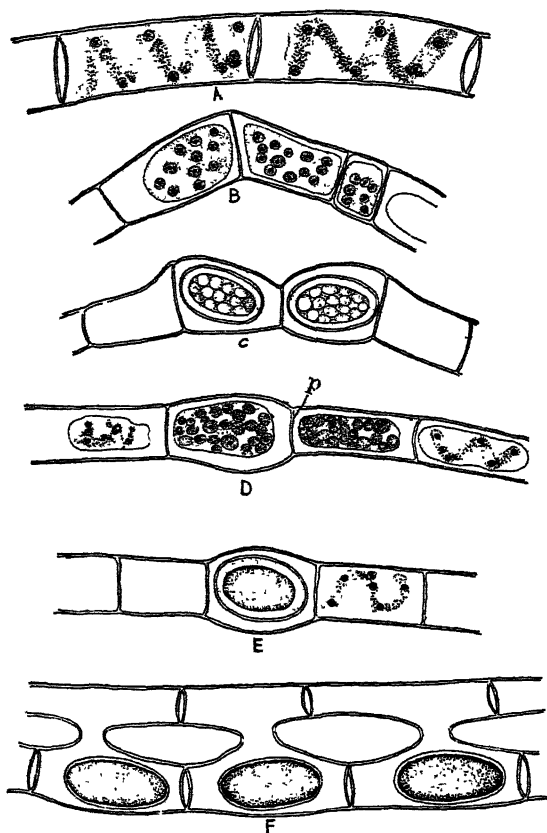


FIG. 35. *Spirogyra parvula* (Trans.) Czurda.

- A.—Shows a vegetative filament with chloroplasts.  
 B.—Shows difference in the size of male and female gametes.  
 C.—Shows a pair of zygospores produced as a result of lateral conjugation.  
 D.—Shows a pore (*p*) in the septum separating the wall from the female cell.  
 E.—Shows the female cell containing a ripe zygospore, and the empty male cell.  
 F.—Shows scalariform conjugation.

All  $\times 310$ .

Both lateral and scalariform conjugation is seen in this species :—

(i) *Lateral conjugation*.—The zygospores occur in pairs at regular intervals, and when they are found singly, they are separated by many

vegetative cells. Lateral conjugation in this species is very interesting. The female cell becomes very much swollen, and its contents become rounded while the male cell is comparatively smaller in size (Fig. B). Thus there is not only a physiological difference between the gametes, but also a morphological one. The contents of both the male and female cells become very much granular and vacuolated. In some cases the female cells are swollen on both sides and present a flask-shaped appearance. The male gamete probably passes into the female cell through a pore in the middle of the cell-wall separating the two cells (Fig. D). The empty male cells may be seen adjoining the female cells containing zygospores (Fig. E).

(ii) *Scalariform conjugation*.—This type of conjugation was seen in a material collected from Siah Baeen in the month of March, 1931. The female cells are clearly swollen (Fig. F).

Zygospores ellipsoid to oval in shape,  $22-26\ \mu$  broad, and  $36-54\ \mu$  long. The zygospore wall is made up of three layers, a smooth and brown exospore, a smooth and bluish-green mesospore, and a light brown endospore.

*Habit*.—Found free-floating in a small fresh-water spring near V. Fatehpur, district Saharanpur, in the middle of February 1936, conjugating exclusively in a lateral fashion. Also collected from Siah Baeen, mixed with *Z. caeruleum* during March 1931, conjugating both in a lateral and scalariform fashion. In the lateral material the vegetative filaments were narrower.

7. *Spirogyra paludosa* Czurda,  
*Op. cit.*, *Die Susswasserflora Mitteleuropas*, Heft 9.

(Fig. 36.)

Vegetative cells  $18-22\ \mu$  broad and 5–8 times as long. There is a single chloroplast in each cell. Septa of the cells plain.

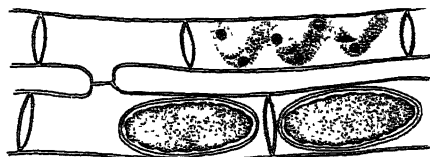


FIG. 36. *Spirogyra paludosa* Czurda.

× 310.

Conjugation scalariform. Female cells containing zygospores slightly swollen. Zygospores ellipsoid, much longer than broad, being  $24-26\ \mu$  broad and  $44-46\ \mu$  long. Exospore hyaline and smooth, mesospore light brown in colour (Fig. 36).

*Habit*.—Found free-floating in a pond at V. Bodal, district Hoshiarpore, in the first week of April 1931.

*Reproduction*.—Conjugation lateral as well as scalariform. In lateral conjugation the zygospores usually occur in pairs. Zygospores oval, 32–36  $\mu$  broad, and 60–70  $\mu$  long. Female cells containing zygospores are not swollen. Exospore hyaline, thick, mesospore brown, and endospore not known (Fig. B). The zygospores produced by lateral conjugation are slightly smaller than those of the type.

Zygospores in forms reproducing by means of scalariform conjugation are bigger, being 42–45  $\mu$  broad, and 70–75  $\mu$  long. Sterile cells with thickened mucilaginous walls frequently alternate with the male cells.

Azygospores may also be seen plentifully, are rounded in appearance and 24–26  $\mu$  in diameter (Fig. C).

*Habit*.—Specimens showing scalariform conjugation were found free-floating in a greenish mass, in a pond in V. Nowshera, district Hoshiarpore, about the middle of October 1930. Also found free-floating in a fresh-water spring at Tahli Sahib, district Hoshiarpore, in the first week of March 1931, reproducing by lateral conjugation.

#### 11. *Spirogyra daedalea* Lagerheim.

*Op. cit.*, Czurda, *Susswasserflora Mitteleuropas*—Zygnemales, Heft 9.

(Fig. 40.)

Vegetative cells 30–38  $\mu$  broad, 6–10 times as long. Septa of the plain type. Each cell with a single spiral chloroplast of 4–5½ turns (Fig. A).

*Conjugation*.—Only scalariform conjugation is known in this species. The female cells are only very slightly swollen, or not swollen at all. Zygospores oval to oval-ellipsoid in shape. Zygospore wall is composed of two layers, a thin, smooth light blue exospore, and a very thick dark brown mesospore. The zygospore wall has an irregularly network-like sculpturing on it. Zygospores are 36–42  $\mu$  broad and 66–92  $\mu$  long and are brownish in colour. Differs from the type in the absence of the horizontal suture on the spore-wall (Fig. B).

*Parthenospores*.—A very interesting form of parthenospore-formation was seen in this species. In a part of two conjugating filaments zygospores of the normal type were seen, while in another part parthenospores were seen in both the male and the female cells, though conjugation canals are completely formed. It seems that in this case the male and female gametes instead of fusing to form zygospores developed independently in their respective cells into parthenospores, due to some unknown reason. The parthenospores developed from the female gametes were much larger in size as compared with those developed from male gametes, and were devoid of any pigmentation,

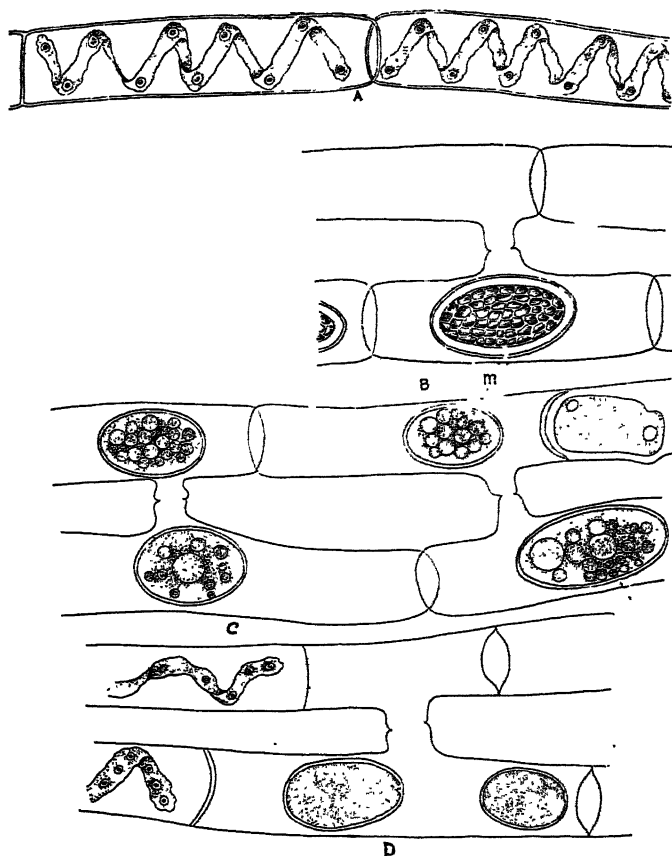


FIG. 40. *Spirogyra dædalea* Lagerheim.

A.—Shows a vegetative filament.

B.—Shows scalariform conjugation with a ripe zygospore with reticulate markings.

C.—Shows parthenospores developed from male (m) and female (f) gametes.

D.—Shows two parthenospores in the female cell.

All  $\times 310$ .

while those developed from the male gametes were coloured brownish-green (Fig. C). Such parthenospores have been reported by Rosenvinge in *S. Grænlantica*.

Another very interesting type of parthenospore-formation was seen in a female cell of one of the two conjugating filaments, the remaining parts of which showed normal conjugation. In this case both the parthenospores, developed respectively from the male and female gamete, were seen in the female cell. In this case it seems, that the male gamete successfully entered the female cell, but instead of fusing with the female gamete developed

independently into a parthenospore. Even in this case, difference in the size of the parthenospore developed from the male and female gametes is noticeable, the former being very much smaller in size as compared with the latter (Fig. D).

*Habit.*—Found free-floating in a fresh-water jhil near Baskhari, tehsil Tanda, district Fyzabad, mixed with *Debarya costata* sp. nov., during the first week of December 1936 and free-floating in Tons Nadi near Akbarpur mixed with *Nodularia spumigena* on 20th March 1937.

12. *Spirogyra Oudhensis* sp. nov.

(Fig. 41.)

Vegetative cells 34–38  $\mu$  broad and 4 to 6 times as long, each containing a single chloroplast of 3–4½ spirals, septa plain.

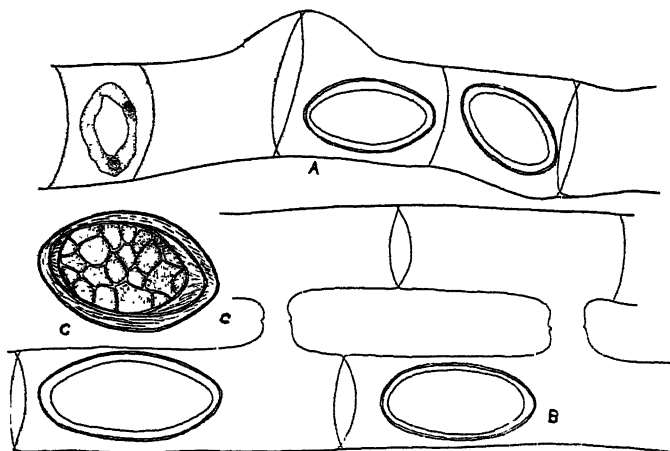


FIG. 41. *Spirogyra Oudhensis* sp. nov.

A.—Shows lateral conjugation.

B.—Shows scalariform conjugation.

C.—Shows the structure of a zygospore. A and B are  $\times 310$ ; C is  $\times 410$ .

*Conjugation.*—In this form conjugation is both lateral and scalariform. Zygospores are oval, 30–40  $\mu$  broad and 55–86  $\mu$  long. Spore-wall bears broad reticulations on surface, and mesospore is separated from the other two layers by a considerable space (Fig. C).

This form resembles *S. dædalea* in the dimension of cells and spores, but differs from it in the presence of lateral conjugation, broad nature of reticulations and peculiar spore layers.

*Habit.*—Found free-floating in a pond near V. Mhow Shivala, district Fyzabad, in October and November 1937.

13. *Spirogyra quadrata* (Hass) Petit.

*Op. cit.*, Borge *Susswasserflora*, Heft 9.

(Fig. 42.)

Vegetative cells  $28-32\ \mu$  in diameter, 3-4 times as long. A single chloroplast in each cell with two to six spirals. Septa replicate.

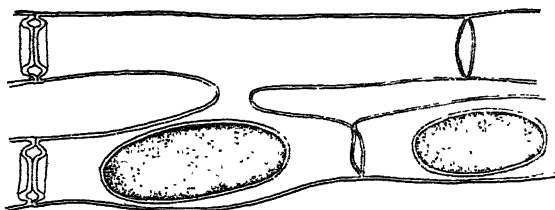


FIG. 42. *Spirogyra quadrata* (Hass) Petit.  
Shows scalariform conjugation with ripe zygospores.  $\times 220$ .

*Reproduction*.—Conjugation scalariform. Fertile cells swollen on both sides. Zygospore-wall smooth. Zygospores ellipsoid-elongated,  $32-42\ \mu$  in diameter,  $2-2\frac{1}{2}$  times as long (Fig. 42).

*Habit*.—Free-floating in a greenish mass of filaments in a fresh-water stream near V. Kiri, district Gurdaspur, in the middle of December 1930.

14. *Spirogyra Goetzei*, Schmidle (1902).

*Op. cit.*, Czurda, *Susswasserflora Mitteleuropas*, Heft 9.

(Fig. 43).

Vegetative cells are  $22-24\ \mu$  broad and 5-7 times as long. Each cell has a single spiral chloroplast bearing numerous conspicuous pyrenoids on it. Septa of the walls are replicate (Fig. A).

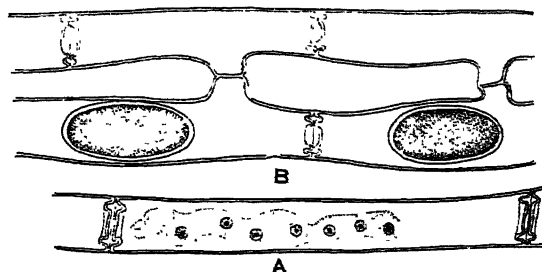


FIG. 43. *Spirogyra Goetzei* Schmidle.

A.—Shows a chloroplast in a vegetative cell.

$\times 310$ .

B.—Shows scalariform conjugation with ripe zygospores.

*Conjugation*.—Only scalariform conjugation is known in this species. Female cells containing zygospores are slightly swollen. Zygospores are

more or less oval in shape. The zygospore wall is composed of two layers only, a thin hyaline exospore, and a brownish mesospore. The zygospores are  $24-28\ \mu$  broad and  $50-56\ \mu$  long (Fig. B).

This form differs from the type in the absence of punctation from the mesospore.

*Distribution.*—This species has so far been reported from lake Nyasa in South Africa.

*Habit.*—Found mixed with *Zygnema giganteum*, *Z. caeruleum* and *Spirogyra parvula* in Siah Baeen, a fresh-water stream in district Jullundar, Punjab, during the second week of March 1931.

15. *Spirogyra Grevilliana* (Hass.) Czurda (1930).

*Op. cit.*, *Susswasserflora Mitteleuropas*, Heft 9.

Vegetative cells  $31-35\ \mu$  broad, 2-4 times as long. Septa replicate. Chromatophore single with 3-5 spirals.

*Reproduction.*—Conjugation lateral. Fruiting cells  $36-42\ \mu$  thick, slightly swollen in the middle. Zygospores broadly ellipsoid,  $28-32\ \mu$  thick and twice as long. Spore-walls smooth.

*Habit.*—Free-floating in a pond near Shahdra, Lahore, in the middle of March 1930. Also collected from Saharanpur in March 1935.

16. *Spirogyra Tandæ* sp. nov.

(Fig. 44.)

Vegetative cells  $16-19\ \mu$  broad, 6-9 times as long, each cell containing a single spiral chloroplast with 3-6 turns, septa replicate.

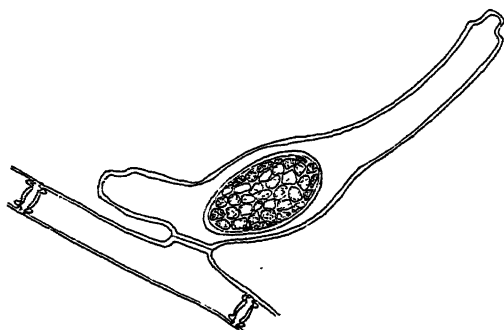


FIG. 44. *Spirogyra Tandæ* sp. nov.

$\times 310$ .

*Conjugation.*—Reproduction takes place by means of scalariform conjugation only. Conjugation canals very short and broad. Female cell cylindrically inflated. Zygospore oval to ellipsoid in shape,  $25-32\ \mu$  broad and

56–62  $\mu$  long. Zygosporc wall is composed of two layers only, a thin and smooth exospore, and a thick mesospore with foveolate reticulations.

*Affinities*.—This alga resembles *S. cylindrica* Czurda in the shape of its cylindricallv inflated fruiting cells, but differs from it in the bigger and longer size of its vegetative cells, its very brief conjugation canals, and reticulations on spore-wall, which is smooth in *S. cylindrica*, hence it is desirable to treat it as a new species.

*Habit*.—Found free-floating in a fresh-water stream near Tanda, district Fyzabad, U.P., on 8th February 1937.

17. *Spirogyra foveolata* (Transeau) Czurda nov. nom.

*Spirogyra inflata* (Vauch) Rab.

*Op. cit.*, Czurda, *Susswasserflora Mitteleuropas*, Heft 9.

Vegetative cells 12–18  $\mu$  thick, 7–10 times as long, septa replicate, chloroplast single with 3–6½ spirals, sometimes almost straight.

*Conjugation*.—Both lateral and scalariform conjugation is seen in this species. A peculiar type of scalariform conjugation in which zygospores are seen in an alternate fashion in opposite cells of the conjugating filaments, known as cross-conjugation was quite commonly seen in the specimens collected from V. Shahpur in 1930. This is the only species so far collected by the author, which shows this peculiar type of conjugation. However the specimens collected from a pond in the Military Grass Farm, Fyzabad, in February 1937 showed only the normal type of scalariform conjugation. Lateral conjugation was also seen in some of the filaments in the latter.

The fruiting cells are inflated on both sides. Zygospores are ellipsoid, 24–30  $\mu$  in diameter, and 48–54  $\mu$  long. Reticulate markings are seen on the surface of the zygospores. The zygosporc wall is composed of two layers only, a smooth, thin and light bluish exospore, and a thick brownish mesospore.

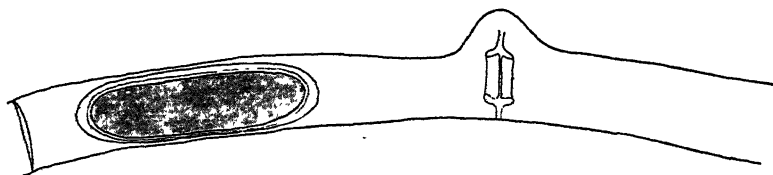
*Habit*.—Found free-floating in a pond at V. Shahpur, district Hoshiarpore Punjab, in the middle of April 1930, from ponds in Saharanpur in April 1935, and Military Grass Farm Fyzabad in February 1937. A common form.

18. *Spirogyra Hassallii* (Jenn.) Petit.

*Op. cit.*, Czurda, *Susswasserflora Mitteleuropas*, Heft 9.

(Fig. 45.)

Vegetative cells 30–32  $\mu$  broad, 6–8 times as long. Two chromatophores. Septa of cells replicate.

FIG. 45. *Spirogyra Hassallii* (Jenn.) Petit.A part of a laterally conjugating filament with a zygospore.  $\times 310$ .

*Conjugation*.—Only lateral conjugation is known in this species. Cells containing the zygospores are only very slightly swollen, unlike typical specimens of the species. Male and female cells always occur in pairs, which are separated by a plain wall. Zygospores ellipsoid, and very much elongated (Fig. 42). Exospore clear, smooth, light-blue in colour. Many of the zygospores contain chytridiaceous fungi.

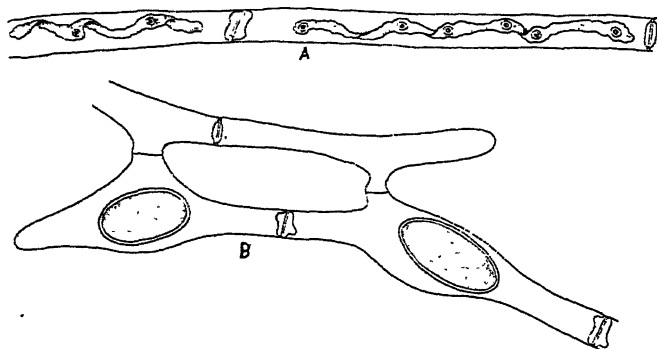
Zygospores  $34-38\ \mu$  broad, and  $64-106\ \mu$  long.

*Habit*.—Found free-floating in a pond at Manglaur, tehsil Roorkee, district Saharanpur, during the third week of February 1935.

19. *Spirogyra unduliseptum* sp. nov.

(Fig. 46.)

Vegetative cells are  $13-18\ \mu$  broad, and 8–12 times as long, each containing a single irregularly spiral chloroplast bearing 3–8 pyrenoids. The septa are peculiarly replicate and look like a parallelogram with the middle lamella as a diagonal, and the longer sides undulating in a wave-like manner (Fig. A).

FIG. 46. *Spirogyra unduliseptum* sp. nov.

A.—Shows a vegetative filament.

B.—Shows a pair of conjugating filaments with ripe zygospores.

$\times 310$ .

*Conjugation*.—Reproduction takes place by means of scalariform conjugation. The female cells are inflated on both sides with the zygospore lying

loosely in the middle. Zygospores are ellipsoid  $20-22\ \mu$  broad and  $40-50\ \mu$  long. The spore-wall bears reticulations on its surface, and is composed of two layers, a thin, smooth and hyaline exospore, and a comparatively thicker yellowish-brown mesospore.

*Affinities.*—The septa of this species differ from those of all known replicate species in their semi-replicate and wavy nature. The only species which can be compared with this form is *S. Narcissiana* Trans reproducing by means of aplanospores only, which are very much elongated as compared with the zygospores of this Indian species.

*Habit.*—Collected from a jhil near Tanda, district Fyzabad, U.P., mixed with a species of *Oedogonium* on 8th February 1937.

20. *Spirogyra lambertiana* Transeau.

*Op. cit.*, Transeau. Tiffany-Taft and Li. *New Species of Zygnemataceæ*, p. 225.

(Fig. 47.)

Vegetative cells  $24-30\ \mu$  broad  $110-260\ \mu$  long, with replicate septa, each cell containing a single chloroplast.

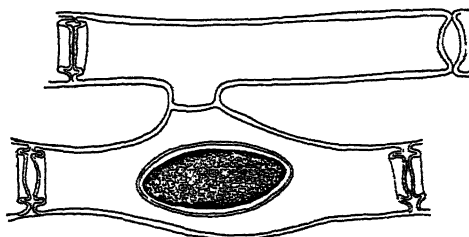


FIG. 47. *Spirogyra lambertiana* Trans.

× 310.

Conjugation scalariform, fertile cells are inflated to  $50-62\ \mu$ . Zygospores are ellipsoid,  $32-36\ \mu$  broad and  $72-80\ \mu$  long. Mesospore is thick, yellow, reticulate.

This form resembles the type in all respects, except that the mesospore lacks an outer wrinkled layer.

*Habit.*—Found, free-floating in Tons Nadi near Akbarpur, district Fyzabad, on 12th February 1938.

21. *Spirogyra gallica* Petit  
var. *bichromatophora* var. nov.

(Fig. 48.)

Vegetative cells  $60-75\ \mu$  broad, and  $96-160\ \mu$  long, each containing two chloroplasts with 4-6 turns.

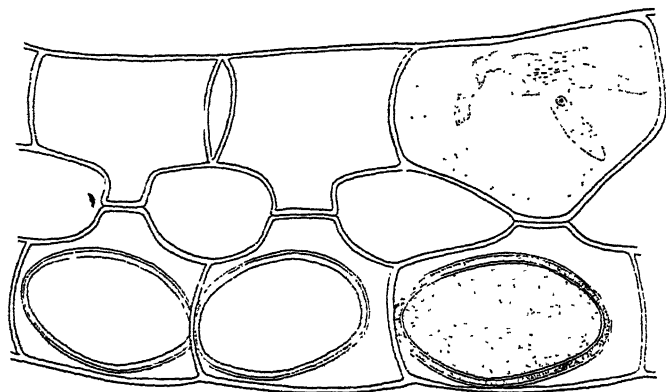


FIG. 48. *Spirogyra gallica* Petit.  
var. *bichromatophora* var. nov.

× 310.

*Conjugation*.—Conjugation scalariform, zygospores ovoid, dark brown in colour,  $54\text{--}60\ \mu$  broad and  $80\text{--}90\ \mu$  long. Spore-wall is differentiated into 3 layers, a thin hyaline exospore, a thick smooth and brown mesospore, and a thin hyaline and smooth endospore.

In one unusual case, the male cell was found to contain some protoplasm and two bits of chloroplasts (Fig. 48) while a completely formed zygospore was seen in the female cell opposite. In this case the whole of the protoplasm has not been utilised in the formation of the male gamete.

*Habit*.—Found free-floating in big brown patches at the sides of Thirua Nadi near Tanda, district Fyzabad, on 21st May 1938.

## 22. *Spirogyra rhizoides* sp. nov.

(Fig. 49.)

This interesting species of *Spirogyra* was found growing in mud covered with a shallow layer of water scarcely an inch in depth, in puddles surrounding the drying banks of Pikia Nadi, a small fresh-water stream, near Rajeh Sultanpur, district Fyzabad, U.P. Unlike the pale yellowish colouration shown by most species of *Spirogyra* this alga was found in the form of a dark brownish mass. In association with it were numerous unicellular green algæ, Desmids, *Mougeotia tenuis* and a species of *Oedogonium*.

Vegetative filaments are usually 2 mm. to  $2\frac{1}{2}$  cm. in length, and have a definite base and apex unlike other species of *Spirogyra*. The basal part bears rhizoids which are fixed in the mud. The apical cells are blunt and spatulate in appearance. The vegetative cells are  $26\text{--}28\ \mu$  broad, have very thick brown walls, and 2 to 3 closely-packed chloroplasts (Fig. A).

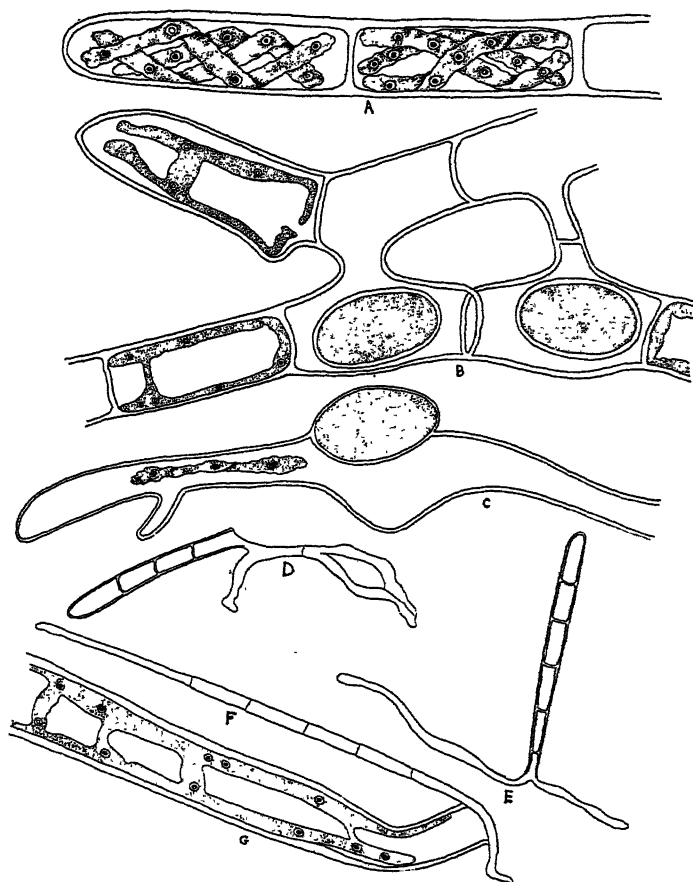


FIG. 49. *Spirogyra rhizoides* sp. nov.

- A.—Shows the apical part of a vegetative filament.  
 B.—Shows scalariform conjugation.  
 C.—Shows an azygospore-like body lodged in a rhizoid.  
 D., E. and F.—Show three different filaments with their rhizoids.  
 G.—Shows a part of a rhizoid with its attenuated chloroplasts.  
 All  $\times 310$  excepting D, E and F which are  $\times 60$ .

*Rhizoids*.—Usually rhizoids are produced from the basal cells, but in some rare cases both ends of the filaments are elongated into rhizoidal structures (Fig. F). In this form, the rhizoids are not short and stumpy structures as in *Spirogyra affinis* Kutz. but are root-like, bifurcated organs, which are extraordinarily long. Other species of *Spirogyra* like *S. bellis* Cleve, and *S. affinis* Kutz. which produce rhizoidal structures were found growing on other water-plants, but never in mud like the present species. The semiterrestrial habit of this alga accounts for the advanced nature of

its rhizoids. The chloroplasts, which are very closely packed in the apical cells, become very much attenuated in the upper part of the rhizoids and the extremities of the rhizoids are usually hyaline. An azygospore was seen lodged in the swollen part of a rhizoid (Fig. C). Zygospore-like structures have been reported by Iyengar,<sup>10</sup> as growing at the extremities of rhizoidal structures in *Mougeotia adnata* Iyeng. but the position in which this azygospore-like body was seen is most peculiar.

*Conjugation*.—Only scalariform conjugation is seen in this alga. The zygospores are usually found in pairs, and in nearly all cases one of the conjugation canals is longer than its neighbour as shown in Fig. B. The zygospores are oval in shape with a tendency towards roundness and are yellowish brown in colour. The fruiting cells are inflated on both sides. The zygospore wall is composed of two layers only, a thin and hyaline exospore, and a thick smooth bluish mesospore. Zygospores are  $36-38\ \mu$  broad, and  $52-58\ \mu$  long.

*Affinities*.—In its peculiar habit, its well-developed rhizoids, its thick cell walls, and its peculiar conjugation canals this species differs from all other known species of *Spirogyra*. The nearest related species is *S. rhizopus* Jao which differs from this species in having cylindrically inflated cell and reticulately sculptured spore-wall.

*Habit*.—Found attached in mud along with *Mougeotia lepus* sp. nov. and a species of *Oedogonium* near the banks of Pikia Nadi near Rajeh Sultanpur, district Fyzabad, U.P., in the last week of November 1936.

23. *Spirogyra dubia* Kutz. var. *brevis*, var. nov.

*Op. cit.*, Borge. *Susswasserflora*, Heft 9.

(Fig. 50.)

Vegetative cells  $27-32\ \mu$  in diameter, 3-7 times as long. Each cell with three chromatophores with  $1\frac{1}{2}$  to  $2\frac{1}{2}$  spirals.

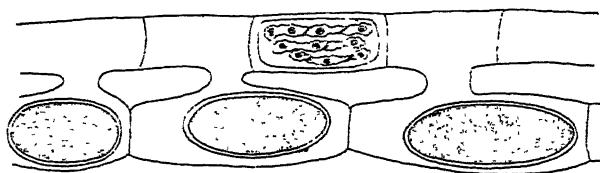


FIG. 50. *Spirogyra dubia*.

Shows two conjugating filaments with zygospores.

× 220.

*Reproduction*.—Only scalariform conjugation is known. Fruiting cells clearly swollen on both the sides. Zygospores are oblong-ellipsoid in shape,  $36-40\ \mu$  in diameter,  $2-2\frac{1}{4}$  times as long. Spore-wall is smooth. Rhizoids

are very commonly given out by many of the cells. It differs from the type in having smaller vegetative cells and having zygospores much longer than broad, hence it is desirable to treat it as a new variety.

*Habit.*—Attached to water-plants or free-floating in ponds. Collected from a pond in V. Jhingran, district Hoshiarpore, in the third week of March, and from Hamira along with *Sirogonium sticticum* in the first week of April 1930.

24. *Spirogyra neglecta* (Hass.) Kutz.

*Op. cit.*, Borge, *Susswasserflora*, Heft 9.

Vegetative cells 50–58  $\mu$  thick. 2–5 times as long, septa plain. Each cell with three chromatophores with 2–2½ spirals.

*Conjugation.*—Conjugation scalariform. Fruiting cells only slightly swollen. Zygospore oval or even rounded, 54–58  $\mu$  in diameter, 1½ times as long as broad. Mesospore smooth and dark brown.

*Habit.*—Found in a blackish mass, free-floating in a pond near V. Bhattian, district Hoshiarpore, in the second week of December 1929.

25. *Spirogyra nitida* (Dillw.) Link.

*Op. cit.*, Borge, *Susswasserflora*, Heft 9.

(Fig. 51.)

Vegetative cells 70–90  $\mu$  in diameter, 1–3 times as long. Septa plain, 3–5 chromatophores with ½ to 1½ spiral in each cell (Fig. A).

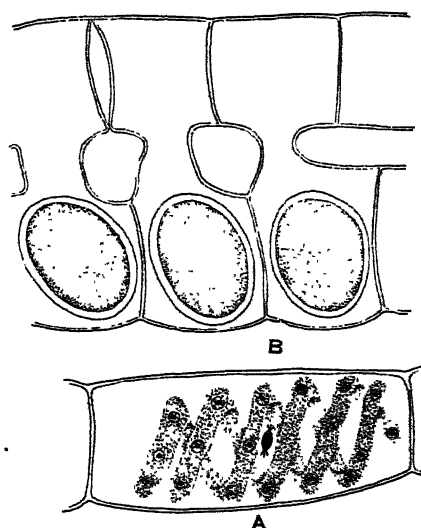


FIG. 51. *Spirogyra nitida* (Dillw.) Link.

A.—Shows a vegetative cell.

B.—Shows ripe zygospores.

× 220.

*Reproduction*.—Conjugation scalariform. Zygospores ellipsoid or even slightly ovoid. Fruiting cells slightly swollen on the outside. Mesospore thick, smooth and dark brown. Zygospores  $50\text{--}55\ \mu$  in diameter,  $1\frac{1}{2}$  times as long (Fig. B). Zygospores are slightly smaller than in the type.

*Habit*.—Free-floating in a pond near Tahli Sahib, district Hoshiarpore, in the second week of October 1929. A very common form.

26. *Spirogyra fluviatilis* Hilse.

[*Spirogyra rivularis* (Hass.) 1 Rab.] *Susswasserflora Mitteleuropas*, Heft 9.

(Fig. 52).

Vegetative cells  $40\text{--}45\ \mu$  broad, 3–6 times as long. Only three irregular chloroplasts were seen in certain cells, as purely vegetative cells were rare and almost all the cells had conjugated in the material examined.

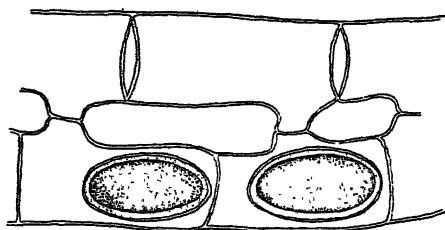


FIG. 52. *Spirogyra fluviatilis* Hilse.

Shows scalariform conjugation with ripe zygospores.  $\times 220$ .

Conjugation scalariform, fertile cells not swollen. Zygospores oval-ellipsoid,  $45\ \mu$  broad,  $70\text{--}75\ \mu$  long. Exospore thin hyaline, mesospore thick dark brown, endospore not known.

*Habit*.—Found free-floating almost filling a pond near V. Bhattian, district Hoshiarpore, in the third week of October 1929. A very common form.

27. *Spirogyra Jacense* sp. nov.

(Fig. 53.)

Vegetative cells are  $44\text{--}56\ \mu$  broad and 2 to  $2\frac{1}{2}$  times as long. There are 4–6 chloroplasts in each cell, and a conspicuous nucleus in the middle. The septa of the cells are plain (Fig. A).

*Conjugation*.—Only scalariform conjugation has been seen in this species. The female cells are only slightly swollen. The zygospores are oval to elliptical in shape and are  $54\text{--}58\ \mu$  broad and  $72\text{--}80\ \mu$  long. The zygospore wall is composed of two layers only, a thin and smooth exospore, and a comparatively thicker brownish mesospore (Fig. B).

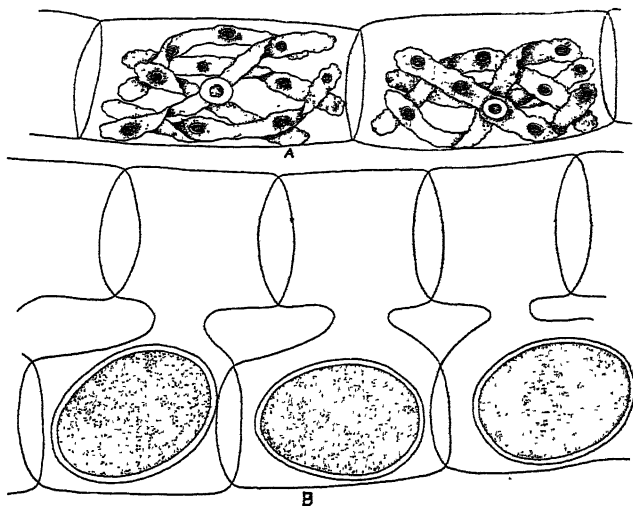


FIG. 53. *Spirogyra Jacense* sp. nov.

A.—Shows a vegetative filament with chloroplasts.

B.—Shows scalariform conjugation and ripe zygospores.

Both  $\times 310$ .

*Affinities.*—This alga resembles *S. Columbiana* Czurda and *S. Occidentalis* (Transeau) Czurda in some features. However it differs from *S. Columbiana* in two important features, firstly the number of chloroplasts, which are 1–3 in the former, while the present form has 4–6 chloroplasts in each cell. Secondly in the former species the mesospore has linear markings on it, while it is smooth in this species. From *S. Occidentalis* (Trans.) Czurda also it differs in the greater number of its chloroplasts, the shape of its zygospores, and the absence of pitting from the mesospore.

*Habit.*—Found free-floating in a fresh-water stream flowing very slowly near Makrahi, district Fyzabad, about the second week of November 1936.

28. *Spirogyra bellis* Cleve.

*Op. cit.*, Borge, *Susswasserflora*, Heft 9.

(Fig. 54.)

Vegetative cells  $60\text{--}65\ \mu$  broad,  $1\frac{1}{2}$  times as long as broad, with plain septa. Each cell with 5–7 chromatophores, closely packed.

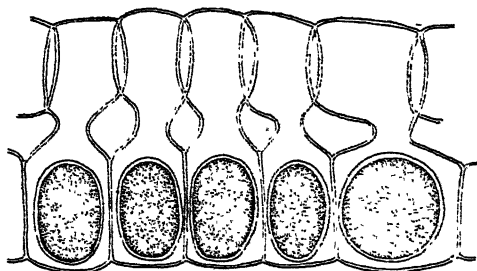


FIG. 54. *Spirogyra bellis* Cleve.

$\times 220$ .

*Reproduction*.—Conjugation scalariform. Zygospores oval or rounded in shape,  $54\text{--}64\ \mu$  in diameter,  $80\text{--}85\ \mu$  long. Mesospore smooth, thick, brownish-yellow in colour. Fruiting cells strongly swollen on both the sides. This alga closely resembles *S. bellis* Cleve in its dimensions but the mesospore is smooth in this case.

*Habit*.—Free-floating in a pond near V. Bodal, district Hoshiarpore, in the second week of March 1930.

29. *Spirogyra Manoramæ* sp. nov.

(Fig. 55.)

Vegetative cells  $80\text{--}100\ \mu$  broad and  $30\text{--}115\ \mu$  long, with thick walls sometimes as thick as  $6\ \mu$ . Each cell contains 7–10 chloroplasts, each with a single turn and bearing numerous pyrenoids on its surface. The nucleus is lens-shaped. Septa plane (Fig. A).

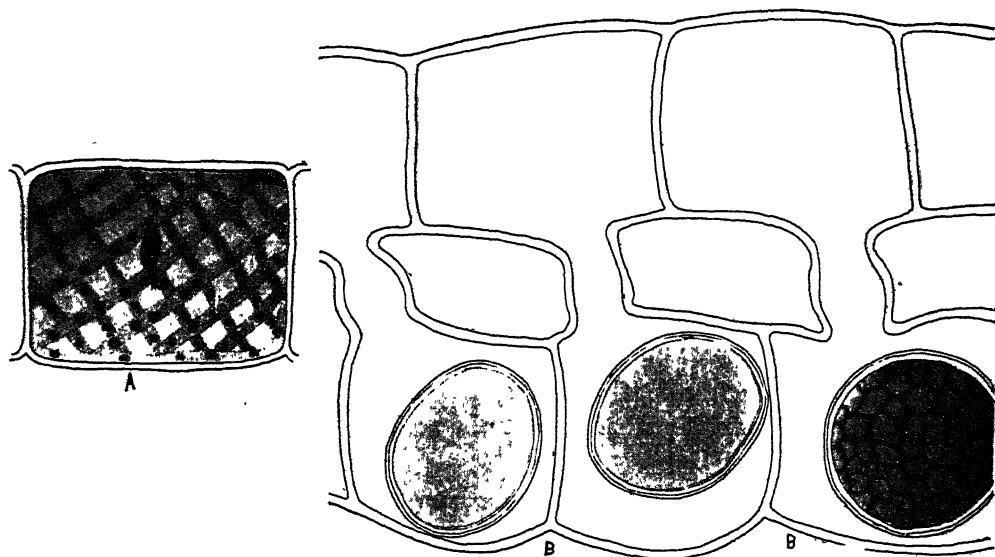


FIG. 55. *Spirogyra Manoramæ* sp. nov.

A.—Shows a vegetative cell with chloroplasts and a nucleus.

B.—Shows conjugation. Mark the polygonal reticulations on the spore-wall of one of the zygospores.  
× 310.

*Conjugation*.—Reproduction by means of scalariform conjugation. Zygospores oval to rounded in shape, lying loosely in the female cells which are inflated on outside. Zygospore wall  $5\ \mu$  thick, and is composed of two layers, a thin smooth exospore, and a thick yellowish brown mesospore with polygonal reticulations. Zygospores are  $65\text{--}82\ \mu$  in diameter (Fig. B).

*Affinities.*—In dimensions of its cells and zygospores this form resembles *S. submaxima* Trans. but it differs from it in having polygonal reticulations on the surface of its zygospores, and its outwardly swollen female cells.

*Habit.*—Found free-floating in Manorama Nadi, a fresh-water stream in district Basti, U.P., on 23rd February 1937.

30. *Spirogyra crassa* (Kutz.).

*Op. cit.*, Borge, *Susswasserflora*, Heft 9.

Vegetative cells 108–118  $\mu$  thick, 1–2 times as long, squarish in shape. Chromatophores 6–10 in number each with a half spiral, each bearing numerous big pyrenoids.

*Reproduction.*—Conjugation scalariform, fruiting cells not swollen. Zygospores brownish, oval to rounded in shape with smooth mesospore. Zygospores 90–110  $\mu$  in diameter, 1–1½ times as long. The alga is slightly smaller than *S. crassa* Kutz. where the zygospores are 144  $\mu$  thick, but in other respects resembles the type.

*Habit.*—Found in a tangle of greenish free-floating mass along with *Oedogonium rivulare* in Shahniwala tank, Dasuya, district Hoshiarpore, in the second week of December 1929. A rather rare form.

31. *Spirogyra submaxima* Transeau var. *inflata* var. nov.

(Fig. 56.)

Vegetative cells are 80–90  $\mu$  broad, and 100–210  $\mu$  long, with thick walls, and each containing 6–8 chloroplasts. In some vegetative cells, the

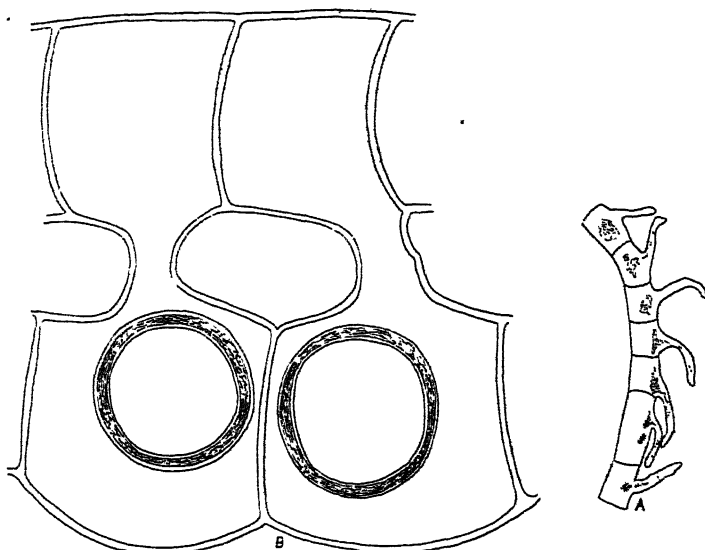


FIG. 56. *Spirogyra submaxima* Trans. var. *inflata* var. nov.

A.—Shows rhizoidal structures.  $\times 60$ .

B.—Shows ripe zygospores.  $\times 310$

conjugation canals were lengthened into rhizoidal structures (Fig. A). This is a remarkable feature, especially because the alga was found free-floating.

*Conjugation.*—Conjugation is scalariform, and fertile cells are inflated on both sides unlike the type. Zygospores are globose  $70-75\ \mu$  in diameter, and brown in colour. Mesospore is thick, brown and smooth.

*Habit.*—Found free-floating in a brownish mass in a fresh-water stream near Gonda-Balrampur Road, on 6th March 1938.

32. *Spirogyra setiformis* (Roth.) Kutz. (1849).

*Op. cit.*, Czurda, *Susswasserflora Mitteleuropas*, Heft 9—*Spirogyra Jugalis* (Dillw.) Kutz.

Vegetative cells  $100-120\ \mu$  in diameter,  $1\frac{1}{2}$  to  $2\frac{1}{2}$  times as long, with plain septa. Each cell with 3-6 chloroplasts making 1-2 spirals.

*Reproduction.*—Conjugation scalariform, fruiting cells not swollen. Zygospores oval or ovoid-rounded,  $80-95\ \mu$  in diameter,  $1\frac{1}{2}$  times as long with a smooth mesospore.

*Habit.*—Free-floating in Siah Baeen near Dasuya, district Hoshiarpore, Punjab, in big masses, in the first week of July 1929. A fairly common form.

*Sirogonium* Kutzing (1843).

Simple filaments, plain end walls. Each cell with 2-9 chloroplasts which are more or less straight, and are found parallel to each other. Conjugation geniculate, conjugation canals very stumpy. One or more sterile cells are cut off from the conjugating cells prior to conjugation, and the female gamete is distinctly bigger in size as compared with the male. Spore-wall is composed of three layers, of which the mesospore may be smooth or sculptured and variously pigmented.

1. *Sirogonium sticticum* Kutz.

*Op. cit.*, Borge, *Susswasserflora*, Heft 9—*Spirogyra stictica* (Engl. Bot.)

Wille, 1884. Czurda, *Susswasserflora Mitteleuropas*, Heft 9.

(Fig. 57.)

Vegetative cells 40–48  $\mu$  broad, 2–4 times as long, 3–5 more or less straight chromatophores each with a number of pyrenoids embedded on it.

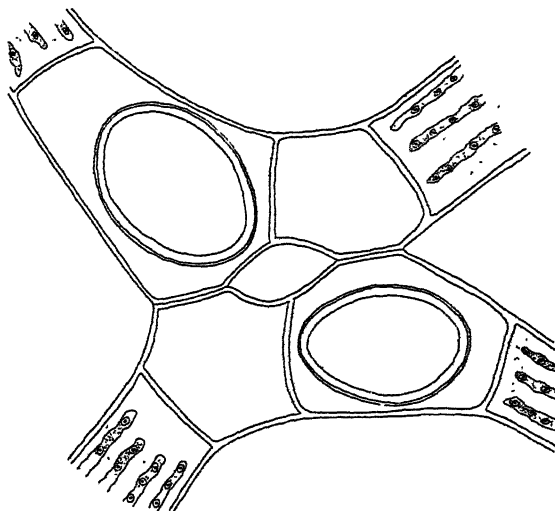


FIG. 57. *Sirogonium sticticum* Kutz.

Shows cross-conjugation.

× 310.

*Reproduction.*—Conjugation geniculate. In one peculiar case cross-conjugation was seen between two filaments (Fig. 58). Zygospores ellipsoid, in some cases bean-shaped, 56–72  $\mu$  broad, 80–90  $\mu$  long, deep orange-yellow in colour.

*Habit.*—Free-floating in a pond. Collected from Hamira and Dhilwan about the middle of March 1930. Also collected from a fresh-water stream in Tanda, district Fyzabad, and Manorama Nadi in district Basti, U.P., in February 1937.

2. *Sirogonium ventersicum* Transeau var. *melanosporum* var. nov.

(Fig. 58.)

Vegetative cells 70–90  $\mu$  broad and 140–260  $\mu$  long, each with 6–9 straight or slightly sinuous chloroplasts.

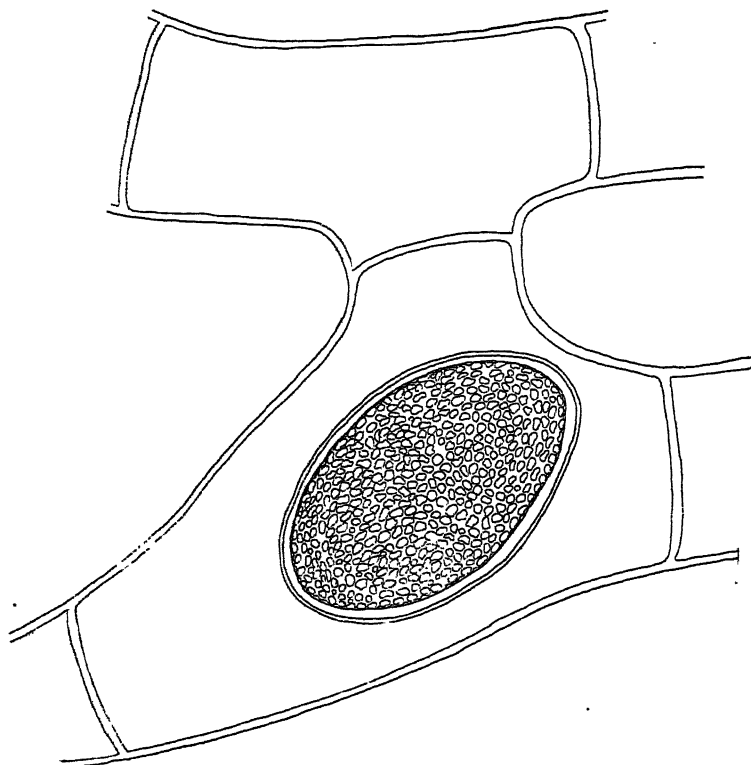


FIG. 58. *Sirogonium ventersicum* Transeau var. *melanosporum* var. nov.  $\times 310$ .

*Conjugation*.—Female cells are inflated to 120–166  $\mu$ . Zygospores are ellipsoid, 90–110  $\mu$  broad and 140–160  $\mu$  long, densely black in colour when fresh. When crushed the verrucose nature of the median spore-wall is clearly revealed.

This form differs from the type in the bigger dimensions of vegetative cells and zygospores, and in the dark black colour of the zygospores.

*Habit*.—Found in vegetative condition in the first week of September and with ripe spores in the end of September and October in a pond near V. Mhow Shivala, district Fyzabad, U.P. A very common species after the rains.

Conclusion.

The author expresses his thanks to his sister-in-law Mrs. Ilse Randhawa for so kindly translating German literature, and to Professor E. N. Transeau for his kindness in examining some of the new species and sending his valuable papers, and Dr. M. O. P. Iyengar for his helpful suggestions and criticism.

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# DIGESTIBILITIES OF THE PROTEINS OF BENGAL GRAM *CICER ARIETINUM* LINN.

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Received August 20, 1938.

PREVIOUS *in vitro* studies of the digestibilities of the proteins of Indian food-stuffs, have been conducted mostly with isolated proteins. From a dietetic point of view such studies have limited significance because foodstuffs always contain a complex mixture of several classes of proteins and the dietician is interested in the fate of these "total proteins".

The results reported in this paper relate to such a study with Bengal gram which forms an important constituent of the diet of all classes of people in India. Globulins from Bengal gram have been isolated and analysed by the Van Slyke's procedure by Niyogi, Narayana and Desai (1931), while Bhagvat (1936) has studied their *in vitro* digestibility. Basu and Mukherjee (1936) have determined the increase in amino nitrogen by the titration procedure of Willstätter when the pulse is subjected to the action of pepsin and trypsin. A comparative study of the digestibility of (1) the isolated globulins, (2) the total proteins and (3) the whole meal has been made.

## *Peptic and Tryptic Digestion of Pulse Meal.*

The seeds, sun dried and cleanly shelled were ground to pass through a 60-mesh sieve. The resulting meal has been used for all the studies described in this paper. Approximate analysis of the meal gave the following results (Table I).

TABLE I.

Moisture	Ash	Ether extractives %	Reducing sugars %	Carbo- hydrates %	Crude protein %	Crude fibre %
9.1	2.4	4.8	0.4	59.8	22.1	1.8

*Peptic digestion.*—The meal (50 gms.) was shaken up with 500 c.c. of 0.05 N HCl (pH about 2.0) and then treated with 50 c.c. of pepsin solution (1 per cent. Pfanstiehl's granular pepsin dissolved in 0.05 N HCl).

The reaction mixture was incubated at 30° C. and shaken at frequent intervals. 20 c.c. aliquots of the mixture were treated with 5 c.c. of 10 per cent. trichloroacetic acid and the total and amino nitrogens determined in the filtrate by the Kjeldahl and Van Slyke methods respectively. An aliquot of 10 c.c. from the incubated mixture was used for a determination of the total nitrogen and a third aliquot (10 c.c.) for the determination of amino nitrogen (Van Slyke). Preliminary experiments to determine the errors of sampling revealed that the maximum variation was less than 1 per cent. The results are calculated on the basis that 11 c.c. of the reaction mixture contain 1 gm. of the meal.

*Tryptic digestion.*—50 gms. of the meal dispersed in 500 c.c. of M/15 phosphate buffer (pH 8.0) and 50 c.c. of trypsin solution (1 per cent. Pfanstiehl's trypsin in the buffer solution) formed the reaction mixture.

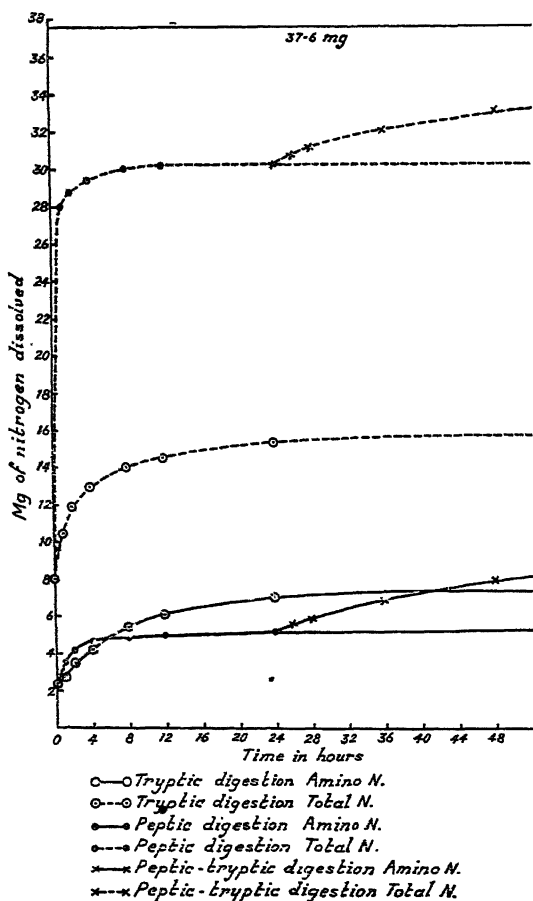


FIG.. 1.

*Pepsin cum trypsin digestion.*—The peptic digest after 24 hours reaction, was adjusted to pH 8 and then subjected to the action of trypsin. The total and amino nitrogens of (1) the reaction mixture and (2) the trichloroacetic acid filtrate were determined. The results are graphically represented in Fig. 1.

The complexities of the trichloroacetic acid precipitable and non-precipitable fractions calculated according to the method of Ranganathan and Sastri (1938) tabulated in Table II.

TABLE II.  
*Complexities (Total N/amino N) of the Fractions.*

Time (hours)	0	1	2	4	8	12	24	
Peptic ..	37.6	25.5	17.8	12.1	12.0	11.7	11.6	Precipitable
Tryptic ..	37.7	27.9	17.7	14.4	9.1	7.9	5.6	
Peptic cum tryptic ..	11.6	..	7.7	6.2	..	4.9	3.7	
Peptic ..	7.9	18.9	11.9	9.4	8.7	8.6	7.6	Non-precipitable
Tryptic ..	7.3	3.5	2.6	1.8	1.7	1.6	1.8	
Peptic cum tryptic ..	7.6	..	7.0	6.6	..	5.4	3.8	

The complexity of the degradation products given in Table II show that in the case of peptic digests, the ratio is above 7, while in the case of tryptic digests, it lies between 1 and 2. These figures give a measure of the extent to which the pulse proteins are degraded under proteoclastic action.

*Digestion of Globulins and Total Proteins.*

It was of interest to ascertain the extent by which such results diverge from those obtained with isolated and purified preparations of "total proteins" and "globulins" when they are subjected to the action of proteoclastic enzymes.

The total globulins were isolated by the method of dialysis employed by Niyogi, Narayana and Desai (*loc. cit.*). The total proteins were isolated by precipitating a clear alkali extract (0.1 per cent. NaOH) of the meal by acidification with acetic acid. The precipitate was redissolved and reprecipitated, then suspended in distilled water and dialysed in cellophane bags

in a running stream of distilled water for three days. The proteins were then separated on the centrifuge, washed with alcohol and ether and finally dried in a desiccator.

The methods employed for following the course of digestion with pepsin, trypsin and pepsin *cum* trypsin have been described above.

The changes in the total and amino nitrogen in the non-protein fraction (not precipitated by trichloroacetic acid) when the pulse meal, globulins and total proteins are subjected to the action of pepsin, trypsin and pepsin *cum* trypsin are graphically represented in Figs. 2 and 3.

Table III gives percentages of nitrogen solubilised (not precipitated by trichloroacetic acid) by proteoclastic enzymes after 24 hours reaction.

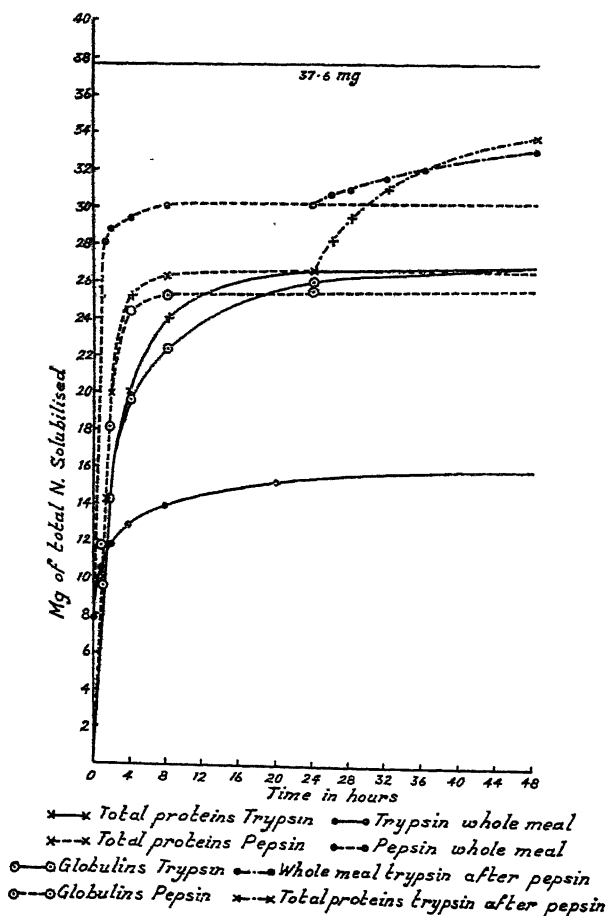


FIG. 2.

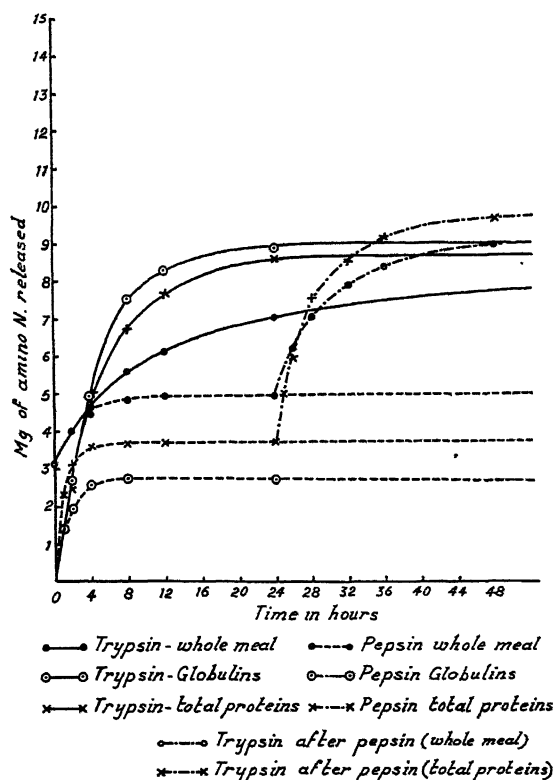


FIG. 3.

TABLE III.

Expressed as percentages of total Nitrogen.

	Pepsin	Trypsin	Pepsin <i>cum</i> trypsin
Total proteins ..	70.8	69.9	88.9
Globulins ..	67.3	71.2	..
Whole meal ..	80.2	43.9	88.1

*Summary.*

1. The hydrolysis of the proteins of Bengal gram has been followed by subjecting the whole meal to peptic, tryptic and peptic *cum* tryptic digestions. It is shown that pepsin releases predominantly polypeptides with a complexity of about 7.

2. Trypsin records a digestibility of 70 per cent. in the case of total proteins and globulins, and only 44 per cent. in the case of whole meal, whereas pepsin records about 70 per cent. in the case of globulins and total proteins, and about 80 per cent. in the case of whole meal. By the successive action of trypsin and pepsin, the total proteins are digested to the extent of nearly 89 per cent., the same being the case with the whole meal.

3. A close study of the tables and graphs reveals that as far as peptic digestion is concerned, there is practically no difference in the digestibilities of total proteins and globulins when present in the seed material or after isolation. In the case of tryptic digestion however, the whole meal records a much lower digestibility.

The author's thanks are due to Mr. M. Sreenivasaya, B.A., F.I.I.Sc., for many helpful suggestions in the course of the work, and also to the Madras Government for the award of a Research Scholarship to one of us (V. R.).

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# THE OEDOGONIALES OF THE UNITED PROVINCES, INDIA—I.\*

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[Communicated by Prof. Y. Bhâradwâja, M.Sc., Ph.D. (Lond.), F.L.S.]

No member of the Oedogoniales has yet been described from the United Provinces. It is therefore desired to record these algæ in a series of papers. The present communication deals with some of the forms collected from the rice fields of Gorakhpur and Benares during last two years. In all the twenty-five forms have been recorded, and out of these six species, six varieties and thirteen forms are new.

## SYSTEMATIC ENUMERATION OF THE SPECIES OBSERVED.

### *Oedogoniales.*

#### Genus *Oedogonium* Link.

#### A. Section *Dioica nannandria*.—

##### 1. *Oedogonium gorakhporensis* sp. nov. (Fig. 1, A-E).

*Idioandrosporous nannandrous*.—Oogonia intercalary or terminal in position, occurring singly or in pairs, rarely in threes, obovoid-ellipsoid in shape; poriferous, pore superior. Oospore globose to sub-globose, not completely filling the oogonium; spore-wall very thick and of three layers, outer layer smooth, inner layers with dentate ribs, also areolate. Suffultory cells swollen; basal cell elongate; apical cell apiculate; vegetative cells cylindrical. Androsporangia in rows of 3-6; nannandria unicellular, ovoid and placed on the oogonium. ■

Lat. cell., veg. fem., 15-22.5  $\mu$ ; long. cell. veg. fem., 150-180  $\mu$ ; lat. cell. veg. masc., 15-18  $\mu$ ; long. cell. veg. masc., 150-180  $\mu$ ; crass. cell. suffult., 30-33  $\mu$ ; crass. oog., 60-67.5  $\mu$ ; long. oog., 90-105  $\mu$ ; crass. oosp., 56.3-60  $\mu$ ; long. oosp., 60  $\mu$ ; lat. androsp., 14-15  $\mu$ ; long. androsp., 13-14.5  $\mu$ ; lat. nannand., 30-37.5  $\mu$ ; long. nannand., 35.5-48.8  $\mu$ .

*Habitat*.—Along with *Spirogyra kundaensis* sp. nov., Kunda Ghat, Gorakhpur; October 19, 1936; November 28, 1937.

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\* From the Department of Botany, Benares Hindu University.

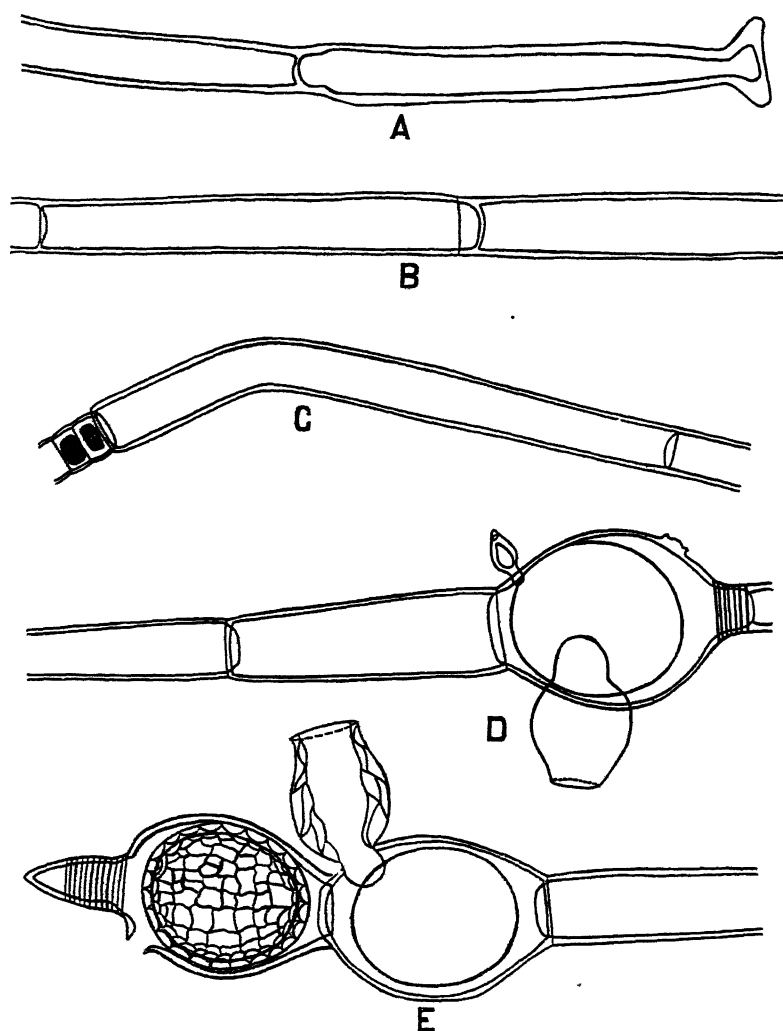


FIG. 1.

A.—A portion of the filament showing the attachment cell, B.—a portion of the filament showing vegetative cells, C.—a filament showing androsporangia, D.—a female filament showing oogonium and dwarf males, and E.—a female filament showing oospore of *Oedogonium gorakhporensis* sp. nov.

All  $\times 346$ .

The alga can only be compared with *Oedogonium Taylorii* Jao (Jao, "Oedogonium in the vicinity of Woods Hole, Massachusetts," *Rhodora*, Vol. 36, 1934, Pl. 286, Figs. 9-12) on account of its nanandrous and idioandrosporous habit, 1-3 oogonia, and superior pore; but, it differs from the same in cylindrical vegetative cells, unicellular and ovoid dwarf males, obovoid ellipsoid oogonia, globose to sub-globose oospores, and in the character of

the spore-walls. It further contrasts with the above alga in possessing bigger vegetative cells, oogonia and oospores and in the position of the dwarf males which is always on the oogonium.

2. *Oedogonium silvaticum* Hallas. Tiffany, *The Oedogoniaceæ*, Ohio, 1934, p. 127, Pl. XLIX, Fig. 472.

var. *idioandrosporum* var. nov. (Fig. 2, A-C).

*Idioandrosporous nannandrous*.—Oogonia intercalary in position, occurring singly, ellipsoid-globose; poriferous, pore superior, Oospores globose, not filling the oogonia; spore-wall thick and smooth. Suffultory cells sub-tumid and swollen; basal cells elongate; apical cells obtuse. Androsporangia in rows of 3-10; nannandria sub-erect on suffultory cells; antheridium exterior.

Lat. cell. veg. fem., 10-13  $\mu$ ; long. cell. veg. fem., 56-70  $\mu$ ; lat. cell., veg. masc., 11-13  $\mu$ ; long. cell. veg. masc., 66-85  $\mu$ ; crass. cell. suffult., 29.7-32  $\mu$ ; crass. oog., 43-45  $\mu$ ; long. oog., 53-55  $\mu$ ; crass. oosp., 36.3-39  $\mu$ ; long. oosp., 36.3-39  $\mu$ ; lat. androsp., 9-10  $\mu$ ; long. androsp., 9-10  $\mu$ ; lat. nannand. stipes., 13.2-15  $\mu$ ; long. nannand. stipes., 36.3-40  $\mu$ .

*Habitat*.—Along with *Spirogyra longata*, Gorakhpur; October 7, 1936; November 25, 1937.

The variety resembles the type in single ellipsoid-globose oogonium, superior pore, globose oospore not filling oogonium, sub-tumid suffultory cell, sub-erect nannandria and external antheridia; but, it differs from the same in smaller vegetative cells and bigger suffultory cells, oogonia, oospores and nannandria and in its idioandrosporous habit.

3. *Oedogonium borisianum* (Le Clerc) Wittrock Hirn, *Monographie und Iconographie der Oedogoniaceen*, 1900, p. 217, Pl. XXXVI, Fig. 223; Tiffany, *op. cit.*, 1930, p. 128, Pl. XLVIII, Fig. 469.

var. *crassa* var. nov. (Fig. 2, D).

*Idioandrosporous nannandrous*.—Oogonia intercalary, occurring singly or in pairs, also in threes, obovoid or quadrangular-ellipsoid; poriferous, pore superior. Oospores ovoid to obovoid, nearly filling the oogonia; spore-wall thin and smooth. Suffultory cells very much swollen; basal cell elongate; terminal cell broadly apiculate. Nannandria very much curved, and placed on the suffultory cell.

Lat. cell. veg. fem., 30-33  $\mu$ ; long. cell. veg. fem., 100-180  $\mu$ ; lat. cell. veg. masc., 30-33  $\mu$ ; long. cell. veg. masc., 135-150  $\mu$ ; crass. cell. suffult., 40-50  $\mu$ ; crass. oog., 55-60  $\mu$ ; long. oog., 60-63  $\mu$ ; crass. oosp., 52-55  $\mu$ ;

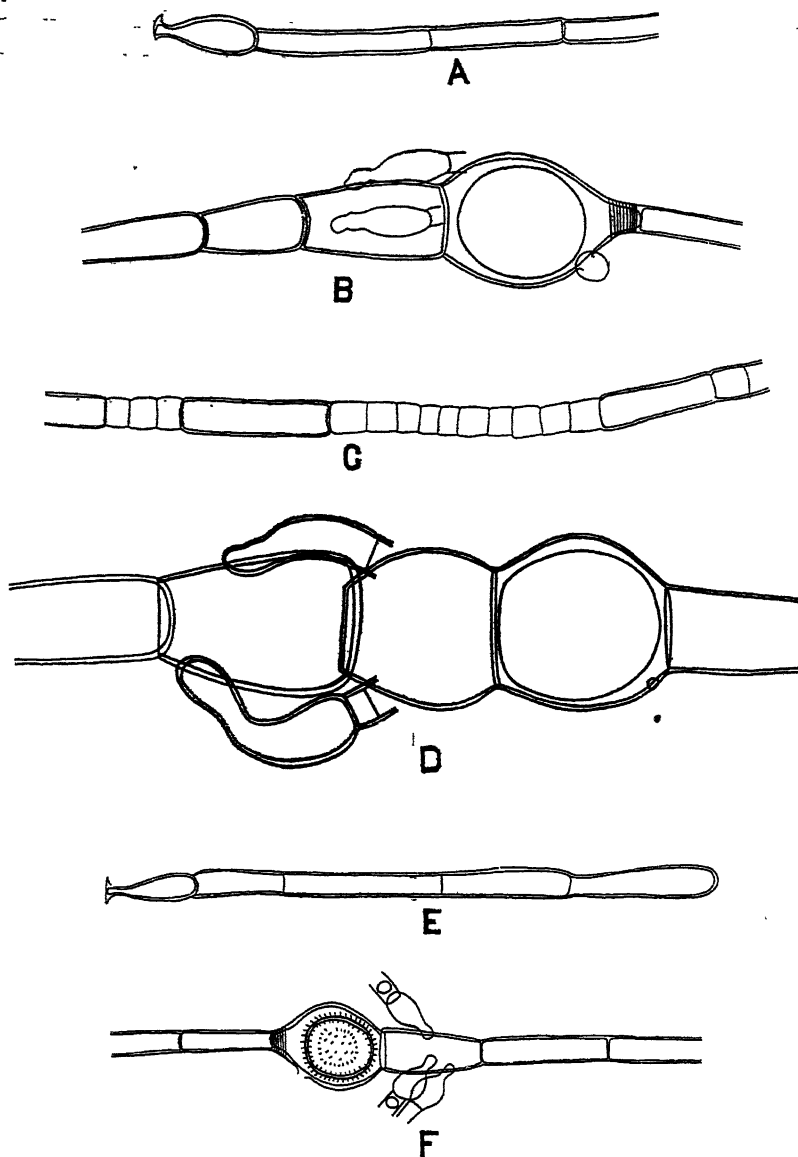


FIG. 2.

A.—A portion of the filament showing the attachment cell, B.—a female filament showing oogonium, oospore and dwarf males, and C.—a filament showing androsporangia of *Oe. silvaticum* Hallas. var. *idioandrosporum* var. nov., D.—a female filament showing oogonia, oospore and dwarf males of *Oe. borisianum* (Le Clerc) Wittrock. var. *crassa* var. nov.; E.—a young filament showing basal and apical cells, and F.—a female filament showing oogonium, oospore and dwarf males of *Oe. armigerum* Hirn. forma. *tenuis* form. nov.

All  $\times 346$ .

long. oosp., 54–58  $\mu$ ; lat. androsp., 26.4–28  $\mu$ ; long. androsp., 20–23  $\mu$ ; lat. nannand. stipes., 15–16  $\mu$ ; long. nannand. stipes., 60–63  $\mu$ ; lat. anth., 15–16  $\mu$ ; long. anth., 9.9–11  $\mu$ .

*Habitat*.—Along with *Oe. sociale* Wittrock. forma *minor* form. nov., *Oe. howardii* West. forma *tenuis* form. nov. and *Oe. geniculatum* Hirn, var. *indicum* var. nov., Gorakhpur; October 27, 1937.

The variety resembles the type in 1–3 intercalary oogonia, superior pore, obovoid oospores, smooth spore-walls, swollen suffultory cells, elongate basal cells, and apiculate terminal cells; but, it differs from the same in very much curved nannandria, oospores nearly filling the oogonia broader vegetative and suffultory cells and bigger oogonia, oospores and dwarf males.

4. *Oedogonium armigerum* Hirn Hirn, *op. cit.*, 1900, p. 203, Pl. XXXIII, Fig. 208; Tiffany, *op. cit.*, p. 133, Pl. XLIII, Fig. 415.

forma. *tenuis* form. nov. (Fig. 2, E–F).

*Nannandrous*.—Oogonia intercalary, occurring singly, subglobose; poriferous, pore superior. Oospores globose, nearly filling oogonia; spore-wall 2-layered, outer layer echinate. Suffultory cells swollen; basal cell elongate; apical cell obtuse; vegetative cells cylindrical. Nannandria curved, and placed on suffultory cells; antheridia exterior, I—?

Lat. cell., 6.6–10  $\mu$ ; long. cell., 50–80  $\mu$ ; crass. cell. suffult., 13.3–14  $\mu$ ; crass. oog., 26.4–28  $\mu$ ; long. oog., 33–36.3  $\mu$ ; crass. oosp., 23–25  $\mu$ ; long. oosp., 23–25  $\mu$ ; lat. nannand. stipes., 9–10  $\mu$ ; long. nannand. stipes., 19.5–21  $\mu$ ; lat. anth. 6–7  $\mu$ ; long. anth. 7–8  $\mu$ .

*Habitat*.—Along with *Spirogyra azygospora* sp. nov., Gorakhpur; October 20, 1936; October 9, 1937.

The form agrees with the type in subglobose oogonia, superior pore, globose oospores with outer echinate wall, swollen suffultory cells and curved nannandria on suffultory cells; but, it differs from the same in the smaller dimensions of all parts.

5. *Oedogonium decipiens* Wittrock Hirn, *op. cit.*, 1900, p. 266, Pl. XLVI, Figs. 283 and 284; Tiffany, *op. cit.*, 1930, p. 145, Pl. LV, Fig. 520.

var. *poriferum* var. nov. (Fig. 3, A).

*Gynandrosporous nannandrous*.—Oogonia intercalary, occurring singly or in pairs, depressed-globose; poriferous, pore superior. Oospores sub-depressed or depressed-globose, almost filling the oogonia; spore-wall thick

and smooth. Suffultory cells unswollen. Androsporangia 1-3, sub-epigynous, hypogynous, or scattered; nannandria 2-celled, usually on the oogonia; antheridium 1, exterior.

Lat. cell., 10-12.3  $\mu$ ; long. cell., 33-40  $\mu$ ; crass. oog., 26-29.7  $\mu$ ; long. oog., 26-30  $\mu$ ; crass. oosp., 24-26  $\mu$ ; long. oosp., 24-26.4  $\mu$ ; lat. androsp., 9.9-10.5  $\mu$ ; long. androsp., 6.6-7.5  $\mu$ ; lat. nannand. stipes., 13.2  $\mu$ ; long. nannand. stipes., 15-16.5  $\mu$ ; lat. anth., 7-8  $\mu$ ; long. anth., 6.6-7  $\mu$ .

*Habitat*.—Along with *Oe. mesospirale* sp. nov., Gorakhpur; October 19, 1937.

The variety resembles the type in intercalary depressed-globose oogonia, depressed globose oospores, smooth spore-wall and unswollen suffultory cells; but, it differs from the same in being poriferous, superior pore, 2-celled nannandria, external antheridia, smaller oogonia and oospores.

#### 6. *Oedogonium mesospirale* sp. nov. (Fig. 3, B-E).

*Idioandrosporous nannandrous*.—Oogonia intercalary, occurring singly, sub-globose to globose; poriferous, pore supra-median. Oospores globose, nearly filling the oogonia; spore-wall of 3 layers, outer layer smooth, middle layer finely granulate and marked by 5-7 spiral ribs united and sometimes anastomosate at the poles, the polar axis always placed in a transverse position, never parallel with the filament, and the inner layer smooth. Suffultory cells unswollen; basal cell elongate; vegetative cells cylindrical. Androsporangia in rows of 5-8; nannandria multicellular, sub-erect or erect, position variable—on suffultory cells, on oogonia or on vegetative cells; antheridia 4, exterior.

Lat. cell. veg. fem., 26-30  $\mu$ ; long. cell. veg. fem., 75-105  $\mu$ ; lat. cell. veg. masc., 26.4-29.7  $\mu$ ; long. cell. veg. masc., 59.4-82.5  $\mu$ ; crass. cell. suffult., 30  $\mu$ ; crass. oog., 52.5-56.3  $\mu$ ; long. oog., 60-75  $\mu$ ; crass. oosp., 45-49  $\mu$ ; long. oosp., 45-52.5  $\mu$ ; lat. androsp., 23-26  $\mu$ ; long. androsp., 9-13.3  $\mu$ ; lat. nannand. stipes., 13.2-16.5  $\mu$ ; long. nannand. stipes., 33-43  $\mu$ ; lat. anth., 9.9-10.2  $\mu$ ; long. anth., 6.6-8.2  $\mu$ .

*Habitat*.—Along with *Oe. decipiens* Wittrock var. *poriferum* var. nov., Gorakhpur; October 19, 1937.

The alga may be compared with *Oe. exospirale* Tiffany on account of its nannandrous and idioandrosporous habit, sub-globose oogonia, globose or sub-globose oospores with spirally ribbed wall and the multicellular

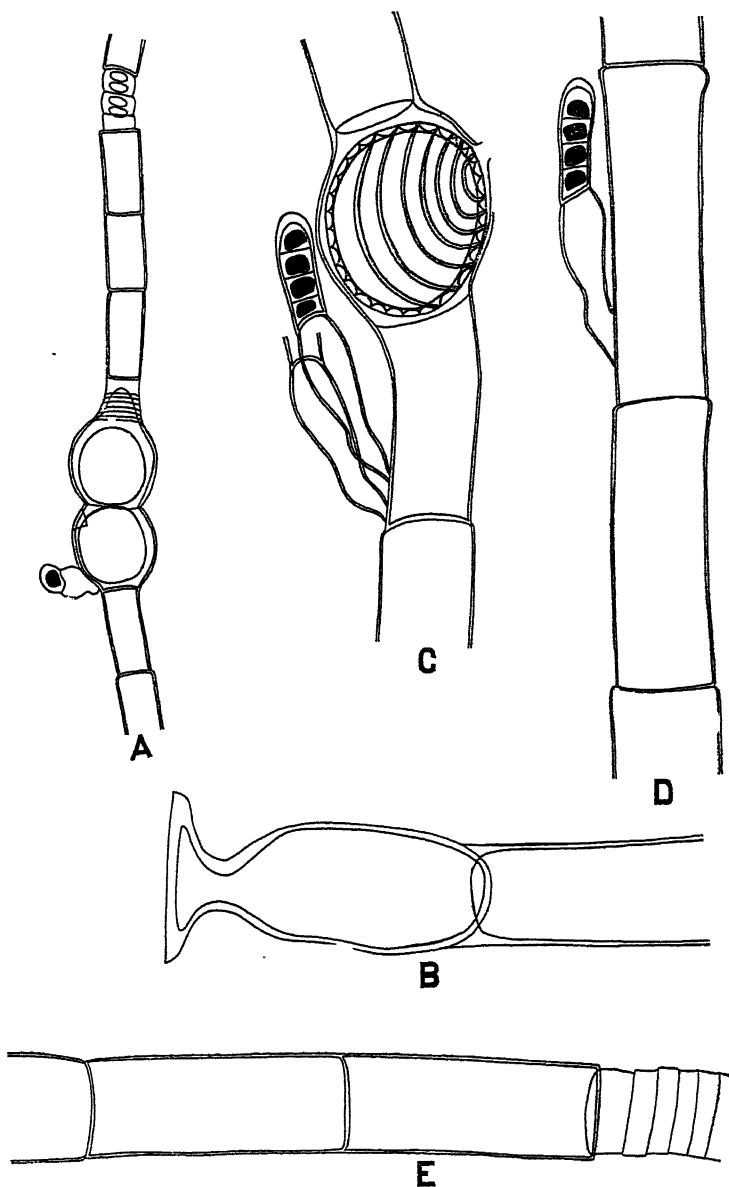


FIG. 3.

A.—A portion of filament showing oogonia, oospores, androsporangia and dwarf male of *Oe. decipiens* Wittrock var. *poriferum* var. nov., B.—a portion of filament showing the attachment cell, C.—a female filament showing oogonium, oospore and dwarf males, D.—a portion of filament showing vegetative cells and dwarf male, and E.—a filament showing androsporangia of *Oe. mesospirale* sp. nov. All  $\times 346$ .

nannandria; but, it differs from the same in the unswollen suffultory cells, supra-median pores, variable position of the nannandria and in the much larger dimensions of all the vegetative and reproductive parts. It further contrasts with the above species in the ribs and spirals being present only on the middle layer of the spore-wall.

7. *Oedogonium spirale* Hirn Hirn, *op. cit.*, 1900, p. 201, Pl. XXXIII, Fig. 206; Tiffany, *op. cit.*, 1930, p. 123, Pl. XLIV, Figs. 427 and 428.

var. *majus* var. nov. (Fig. 4, A-C).

*Idioandrosporous nannandrous*.—Oogonia intercalary, occurring singly, sub-globose or obovoid-globose; poriferous, pore median. Oospores globose or sub-globose, nearly filling the oogonia; spore-walls double, outer layer with 4-7 spiral ribs, anastomosing, united at the poles, inner layer smooth. Suffultory cells unswollen; basal cell elongate. Androsporangia in rows of 2-3; nannandria generally multicellular, sub-erect or erect, sometimes unicellular and ovoid, on suffultory cells or on oogonia; antheridia 4, exterior.

Lat. cell. veg. fem., 37.5-42  $\mu$ ; long. cell. veg. fem., 150-165  $\mu$ ; lat. cell. veg. masc., 32-38  $\mu$ ; long. cell. veg. masc., 132-140  $\mu$ ; crass. oog., 64-67.5  $\mu$ ; long. oog., 75-90  $\mu$ ; crass. oosp., 60  $\mu$ ; long. oosp., 60  $\mu$ ; lat. androsp., 30-33  $\mu$ ; long. androsp., 14-16  $\mu$ ; lat. nannand. stipes., 13-15  $\mu$ ; long. nannand. stipes., 55-60  $\mu$ ; lat. anth., 9-11  $\mu$ ; long. anth., 6-8  $\mu$ ; lat. nannand. ovoid., 15-16.5  $\mu$ ; long. nannand. ovoid., 15-18  $\mu$ .

*Habitat*.—Gorakhpur; October 19, 1937.

The variety agrees with the type in the single sub-globose or obovoid-globose oogonium, median pores, globose or sub-globose oospores with a double spirally-ribbed wall, and multicellular nannandria; but, it differs from the same in sometimes unicellular ovoid nannandria and larger dimensions of both vegetative and reproductive parts.

8. *Oedogonium mirandrium* Skuja Tiffany, *op. cit.*, 1930, p. 147, Pl. LIV, Fig. 514.

forma *apiculata* form. nov. (Fig. 4, D-E).

*Idioandrosporous nannandrous*.—Oogonia intercalary, occurring singly, sub-pyriform globose; operculate, division superior. Oospores globose, quite filling oogonia; spore-wall thin and smooth. Suffultory cells slightly swollen; basal cell elongate; apical cell apiculate. Androsporangia in rows of 5-10; nannandria unicellular and ovoid, placed on oogonia.

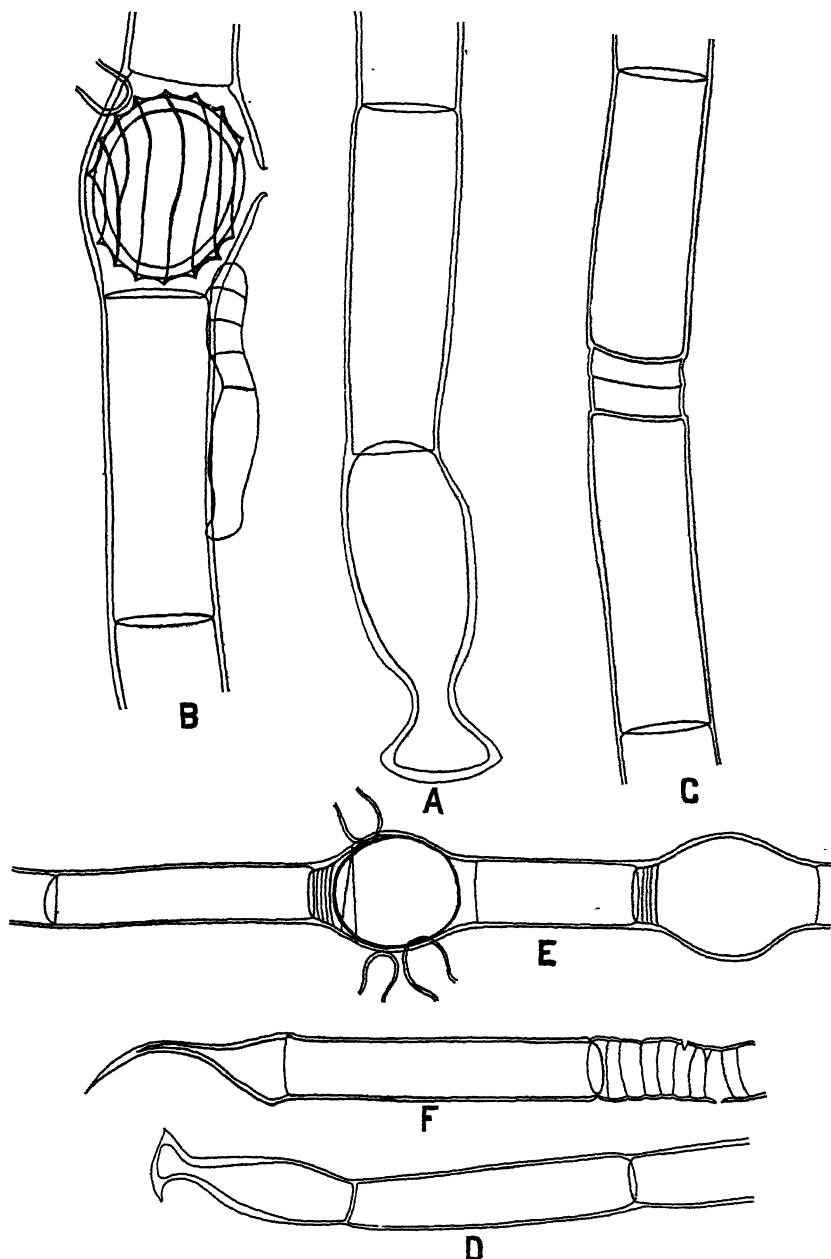


FIG. 4.

A.—A portion of filament showing the attachment cell, B.—a female filament showing oogonium, oospore and dwarf males, and C.—a portion of filament showing androsporangia of *Oe. spirale* Hirn. var. *majus* var. nov.; D.—a portion of filament showing the attachment cell, E.—a female filament showing oogonia, oospore and dwarf males, and F.—a portion of filament showing apical cell and androsporangia of *Oe. mirandrium* Skuja. forma *apiculata* form, nov.

All  $\times 346$ .

Lat. cell. veg. fem.,  $16.5-19.8\ \mu$ ; long. cell. veg. fem.,  $92.4-105\ \mu$ ; lat. cell. veg. masc.,  $16.5-19.8\ \mu$ ; long. cell. veg. masc.,  $90-115\ \mu$ ; crass. cell. suffult.,  $19.8-23.1\ \mu$ ; crass. oog.,  $39.6-43\ \mu$ ; long. oog.,  $45-50\ \mu$ ; crass. oosp.,  $37-40.5\ \mu$ ; long. oosp.,  $39.6-41\ \mu$ ; lat. androsp.,  $18.5-20\ \mu$ ; long. androsp.,  $5-6.6\ \mu$ ; lat. nannand.,  $15-16.5\ \mu$ ; long. nannand.,  $15-18\ \mu$ .

*Habitat*.—Gorakhpur; October 19, 1936; October 7, 1937.

The form agrees with the type in single sub-pyriform globose oogonia, operculate supra-median or nearly superior division, globose oospore, smooth spore-walls, and unicellular ovoid nannandria and elongate basal cell; but, it differs from the same in the apiculate apical cell, bigger oogonia and smaller dwarf males.

9. *Oedogonium longatum* Kuetzing Hirn, *op. cit.*, 1900, p. 239, Pl. XL, Fig. 248; Heering, in Pascher's *Süsswasser-Flora Deutschlands, Österreichs und Der Schweiz*, Heft 6, 1914, p. 182, Fig. 249; Tiffany, *op. cit.*, 1930, p. 148, Pl. LVIII, Fig. 563.

*Forma* (Fig. 5, A).

*Gynandrosporous nannandrous*.—Oogonia intercalary, occurring singly, ellipsoid; operculate, divisions superior. Oospores sub-globose to ellipsoid, nearly filling oogonia, spore-walls thick and smooth. Suffultory cells unswollen; basal cell elongate; apical cell obtuse. Androsporangia 1-2; nannandria on oogonia; antheridium 1, exterior.

Lat. cell.,  $4-5\ \mu$ ; long. cell.,  $39.6-49.5\ \mu$ ; crass. oog.,  $15-17\ \mu$ ; long. oog.,  $24-27\ \mu$ ; crass. oosp.,  $14-16\ \mu$ ; long. oosp.,  $17-19\ \mu$ ; lat. androsp.,  $3.3-4.5\ \mu$ ; long. androsp.,  $3.3\ \mu$ ; lat. nannand. stipes.,  $4-5\ \mu$ ; long. nannand. stipes.,  $10-13.2\ \mu$ ; lat. anth.,  $3.3-4\ \mu$ ; long. anth.,  $4-5\ \mu$ .

*Habitat*.—Along with *Oe. tapeinosporum* Wittrock Benares; November 15, 1937.

The form differs from the type in possessing longer cells and bigger oogonia.

#### B. Section *Dioica macrandria*.

10. *Oedogonium sociale* Wittrock Hirn, *op. cit.*, 1900, p. 79, Pl. II, Fig. 12; Tiffany, *op. cit.*, 1930, p. 67, Pl. XI, Fig. 109.

forma *kanwa'nse* form. nov. (Fig. 5, B-C).

Dioecious, macrandrous; oogonia intercalary, occurring singly or in pairs, sub-globose; poriferous, pore superior or almost so. Oospores

globose or sub-globose, quite filling the oogonia; spore-walls thick and smooth. Antheridia in rows of 4-5; sperms 2, division horizontal.

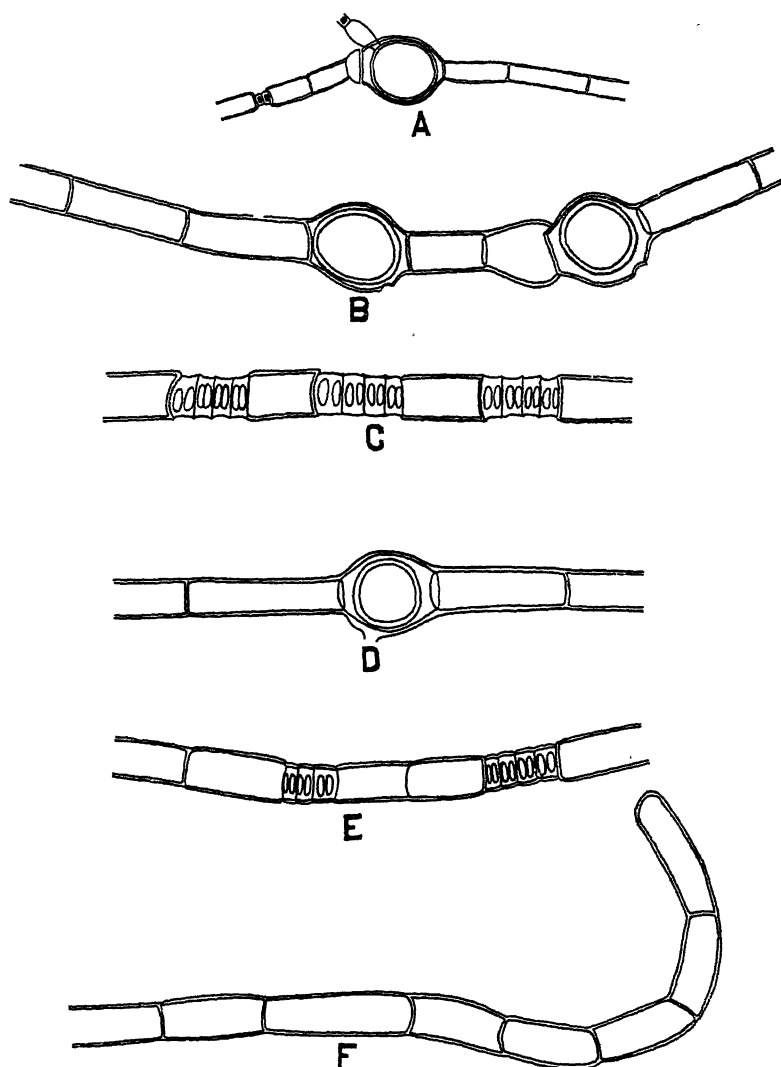


FIG. 5.

A.—A female filament showing oogonium, oospore, antheridia and dwarf male of *Oe. longatum* Kuetzing *Forma*, B.—a female filament with oogonia, oospores, and C.—a portion of filament showing antheridia of *Oe. sociale* Wittrock *forma kanwaense* form. nov., D.—a female filament with oogonium and oospore, E.—a portion of filament showing antheridia, and F.—a portion of filament showing apical cell of *Oe. sociale* Wittrock *forma minor* form. nov.

All  $\times 346$ .

Lat. cell. veg. fem.,  $13.2-14.5\ \mu$ ; long. cell. veg. fem.,  $26.4-55\ \mu$ ; lat. cell. veg. masc.,  $13.2-14.5\ \mu$ ; long. cell. veg. masc.,  $20-30\ \mu$ ; crass. oog.,  $29.7-32\ \mu$ ; long. oog.,  $33-34.5\ \mu$ ; crass. oosp.,  $29.7-31\ \mu$ ; long. oosp.,  $29.7-31\ \mu$ ; lat. anth.,  $11.5-13\ \mu$ ; long. anth.,  $6.6-8\ \mu$ .

*Habitat*.—Kanwa tank, Benares; November 30, 1937.

The form agrees with the type in single sub-globose oogonium, globose or sub-globose oospores with smooth spore-wall and 4-5 antheridia and 2 sperms with horizontal division; but, it differs from the same in superior spore, narrower and smaller cells, oogonia and oospores. It further differs in possessing smaller antheridia.

11. *Oedogonium sociale* Wittrock Hirn, *op. cit.*, 1900, p. 79, Pl. II, Fig. 12; Tiffany, *op. cit.*, 1930, p. 67, Pl. XI, Fig. 109.

forma *minor* form. nov. (Fig. 5, D-F).

Dioecious, macrandrous; oogonium intercalary, occurring singly, sub-globose; poriferous, pore supra-median. Oospores globose, not completely filling oogonia; spore-wall thick and smooth, suffultory cells unswollen; apical cells obtuse. Antheridia in rows of 2-4; sperms 2, division horizontal.

Lat. cell. veg. fem.,  $13.2-15\ \mu$ ; long. cell. veg. fem.,  $43-50\ \mu$ ; lat. cell. veg. masc.,  $13.2-14.5\ \mu$ ; long. cell. veg. masc.,  $36-42\ \mu$ ; crass. oog.,  $26.4-30\ \mu$ ; long. oog.,  $33-36.3\ \mu$ ; crass. oosp.,  $23-26.4\ \mu$ ; long. oosp.,  $23-26.4\ \mu$ ; lat. anth.,  $11-13.2\ \mu$ ; long. anth.,  $5-6.6$ .

*Habitat*.—Gorakhpur; October 7, 1937.

The form agrees with the type in the single intercalary sub-globose oogonium, globose oospores with a smooth spore-wall and in two sperms with a horizontal division; but, it differs from the same in its supra-median pore and very much smaller size of all parts. It further contrasts in oospores not completely filling oogonia.

12. *Oedogonium lautumniarium* Wittrock Hirn, *op. cit.*, 1900, p. 92, Pl. V, Fig. 27; Tiffany, *op. cit.*, 1930, p. 72, Pl. XIV, Figs. 132 and 133.

forma *tenuis* form. nov. (Fig. 6, A-B).

Dioecious, macrandrous; oogonium intercalary, occurring singly or in pairs, sub-obovoid-globose; poriferous, pore superior. Oospores sub-globose, filling oogonia; spore-walls thick and smooth. Antheridia in rows of 2-4; sperms 2, division horizontal.

Lat. cell. veg. fem.,  $19.8-23\ \mu$ ; long. cell. veg. fem.,  $23-35\ \mu$ ; lat. cell. veg. masc.,  $19.8-23\ \mu$ ; long. cell. veg. masc.,  $19.8-38\ \mu$ ; crass. oog.,  $33-37\ \mu$ ; long. oog.,  $29.7-34\ \mu$ ; crass. oosp.,  $30-33\ \mu$ ; long. oosp.,  $27-32\ \mu$ ; lat. anth.,  $18-19.8\ \mu$ ; long. anth.,  $3.5-6.6\ \mu$ .

*Habitat*.—Along with *Oe. intermedium* Wittrock forma *tenuis* form. nov., Gorakhpur; October 15, 1937.

The form resembles the type in single sub-obovoid-globose oogonium, sub-globose oospores with smooth spore-wall; but, it differs from the same in slightly broader and much smaller cells, oogonia and oospores. It further contrasts in having constant superior pore and thickened spore-walls.

13. *Oedogonium areolatum* Lagerheim Hirn, *op. cit.*, 1900, p. 105, Pl. VII, Fig. 45; Tiffany, *op. cit.*, 1930, p. 94, Pl. XXVIII, Figs. 248-250.

forma *ellipso sporum* form. nov. (Fig. 6, C-D).

Dioecious, macrandrous; oogonia intercalary, occurring singly or in threes, ellipsoid-ovoid; poriferous, pore superior. Oospores of the same shape as the oogonia, filling them; spore-wall thick and of three layers, outer and inner layers smooth and middle layer areolate. Antheridia in rows of 5-20; sperms 2, division vertical.

Lat. cell. veg. fem.,  $16.5-19.8\ \mu$ ; long. cell. veg. fem.,  $105-132\ \mu$ ; lat. cell. veg. masc.,  $16.5-17.5\ \mu$ ; long. cell. veg. masc.,  $105-132\ \mu$ ; crass. oog.,  $46.2-49.5\ \mu$ ; long. oog.,  $62.7-69.3\ \mu$ ; crass. oosp.,  $45-49\ \mu$ ; long. oosp.,  $56.1-59.4\ \mu$ ; lat. anth.,  $14-16.5\ \mu$ ; long. anth.,  $7-9.9\ \mu$ .

*Habitat*.—Along with *Oe. mesodentatum* sp. nov., Kunda Ghat, Gorakhpur; October 7, 1937.

The form is characterised by having ellipsoid oogonia and oospores and more antheridia in a row.

14. *Oedogonium franklinianum* Wittrock Hirn, *op. cit.*, 1900, p. 88, Pl. II, Fig. 18; Tiffany, *op. cit.*, 1930, p. 71, Pl. XIII, Fig. 128.

forma *crassa* form. nov. (Fig. 6, E-F).

Dioecious, macrandrous; oogonia intercalary, occurring singly or in pairs, sub-globose; poriferous, supra-median pore. Oospores globose, almost filling oogonia; spore-wall thick and smooth. Antheridia in rows of 2-4; sperms 2, division horizontal.

Lat. cell. veg. fem.,  $14-15\ \mu$ ; long. cell. veg. fem.,  $39.6-50\ \mu$ ; lat. cell. veg. masc.,  $13.2-14.5\ \mu$ ; long. cell. veg. masc.,  $39.6-50\ \mu$ ; crass. oog.,

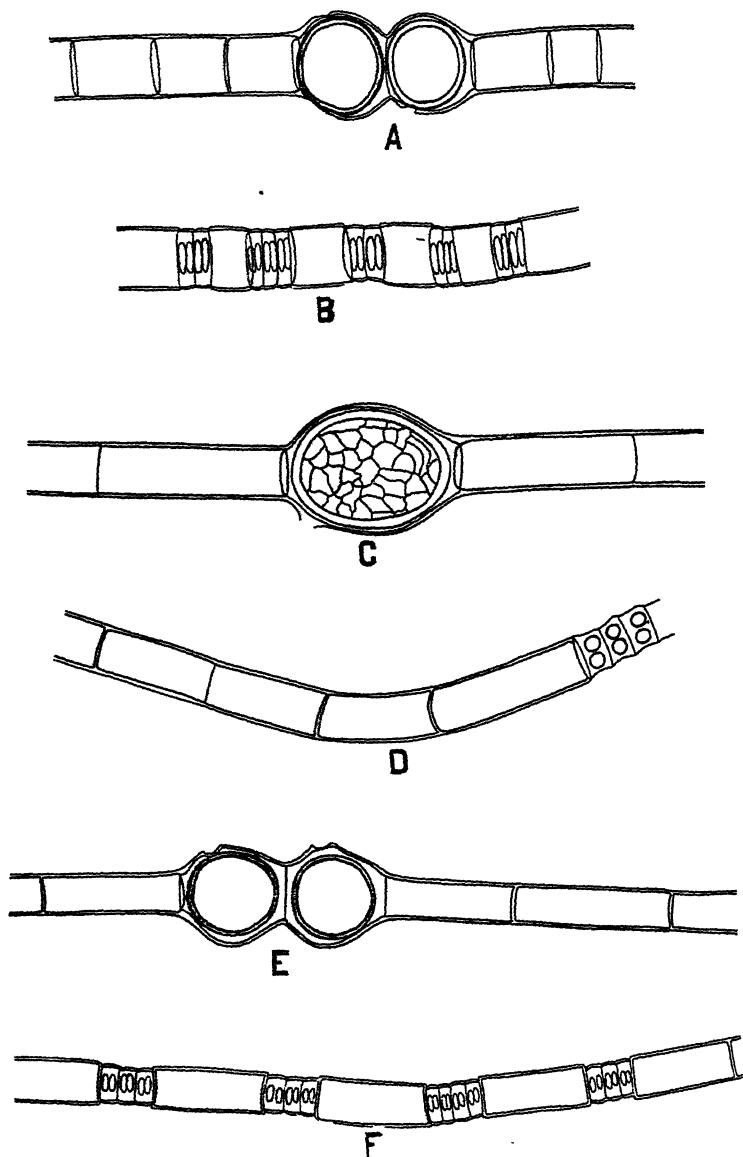


FIG. 6.

A.—A female filament with oogonia and oospores, and B.—a portion of filament showing antheridia of *Oe. lautumnarum* Wittrock forma *tenuis* form. nov., C.—a female filament with oogonium and oospore, and D.—a portion of filament with antheridia of *Oe. areolatum* Lagerheim forma *ellipsosporum* form. nov., E.—a female filament with oogonia and oospores, and F.—a portion of filament with antheridia of *Oe. franklinianum* Wittrock forma *crassa* form. nov.

All  $\times 346$ .

30–33.3  $\mu$ ; long. oog., 30–35  $\mu$ ; crass. oosp., 29.7–33.3  $\mu$ ; long. oosp., 29.7–33.3  $\mu$ ; lat. anth., 13–14  $\mu$ ; long. anth., 7–9.9  $\mu$ .

*Habitat*.—Gorakhpur; October 19, 1936; November 5, 1937.

The form differs from the type in having broader cells, oogonia, oospores and antheridia.

15. *Oedogonium capilliforme* Kuetzing; Wittrock Hirn, *op. cit.*, 1900, p. 107, Pl. VIII, Fig. 49; Tiffany, *op. cit.*, 1930, p. 82, Pl. XIX, Figs. 172 and 173.

*Forma* (Fig. 7, A–B).

Lat. cell. veg. fem., 39.6–42  $\mu$ ; long. cell. veg. fem., 165–211  $\mu$ ; lat. cell. veg. masc., 39.6–42  $\mu$ ; long. cell. veg. masc., 165–203  $\mu$ ; crass. oog., 52.8–56  $\mu$ ; long. oog., 66–76  $\mu$ ; crass. oosp., 48–50  $\mu$ ; long. oosp., 56–60  $\mu$ ; lat. anth., 35–39.6  $\mu$ ; long. anth., 5–9  $\mu$ .

*Habitat*.—Gorakhpur; November 5, 1937.

The form differs from the type in possessing bigger vegetative cells, oogonia and oospores.

16. *Oedogonium ellipsosporum* sp. nov. (Fig. 7, C–F).

Dioecious, macrandrous; oogonia 1–5, intercalary, sub-oblong-ellipsoid; poriferous, pore superior. Oospores sub-globose-ellipsoid, almost filling oogonia; spore-walls thin and smooth. Suffultory cells slightly swollen; basal cell elongate; apical cell obtuse; vegetative cells cylindrical. Antheridia in rows of 7–10; sperms 2, division vertical.

Lat. cell. veg. fem., 11.6–16.5  $\mu$ ; long. cell. veg. fem., 69.3–82.5  $\mu$ ; lat. cell. veg. masc., 13.2–15  $\mu$ ; long. cell. veg. masc., 63–70  $\mu$ ; crass. cell. suffult., 17–18.5  $\mu$ ; crass. oog., 33.3–35  $\mu$ ; long. oog., 59–4.61  $\mu$ ; crass. oosp., 29.7–31  $\mu$ ; long. oosp., 43–45  $\mu$ ; lat. anth., 13.2–15  $\mu$ ; long. anth., 4.5–5.2  $\mu$ .

*Habitat*.—Ramgarh Tal, Gorakhpur; October 19, 1937.

The alga may be compared with *Oe. grande* Kuetzing; Wittrock on account of the 1–5 oogonia, superior pore, smooth spore-wall, 10-seriate antheridia and 2 sperms with vertical divisions; but, it differs from the same in sub-oblong-ellipsoid oogonia, ellipsoid oospores, male filaments of nearly the same dimensions as the female filament, and in much smaller dimensions of all parts.

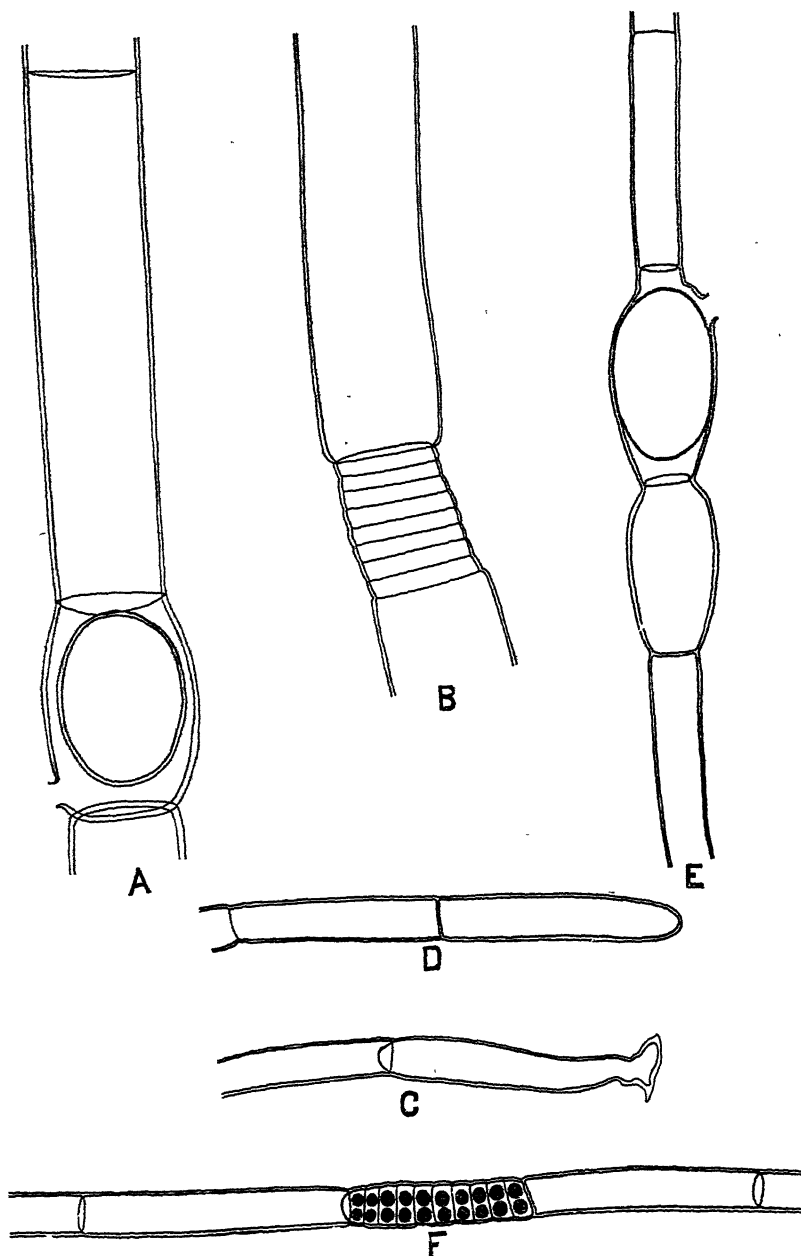


FIG. 7.

A.—A female filament with oogonium and oospore, and B.—a portion of filament showing antheridia of *Oe. capilliforme* Kuetzing; Wittrock *Forma*; C.—a portion of filament showing basal cell, D.—a portion of filament showing apical cell, E.—a female filament with oogonia and oospore, and F.—a portion of filament showing antheridia of *Oe. ellipsosporum* sp. nov.

All  $\times 346$ .

17. *Oedogonium mesodentatum* sp. nov. (Fig. 8, A-B).

Dioecious, macrandrous; oogonia intercalary, occurring singly or in pairs, also in threes, obovoid to sub-ellipsoid; poriferous, pore superior. Oospores obovoid to sub-ellipsoid, filling oogonia; spore-walls 3-layered, outer layer smooth, middle layer areolate with dentate ribs and the inner layer smooth, suffultory cell unswollen; basal cell elongate; vegetative cells cylindrical. Antheridia in rows of 2-5; sperms 2, division horizontal.

Lat. cell. veg. fem.,  $13.2-14\ \mu$ ; long. cell. veg. fem.,  $55-60\ \mu$ ; lat. cell. veg. masc.,  $12-13.2\ \mu$ ; long. cell. veg. masc.,  $56-60\ \mu$ ; crass. oog.,  $30-33.3\ \mu$ ; long. oog.,  $49.5-60\ \mu$ ; crass. oosp.,  $26.4-29.6\ \mu$ ; long. oosp.,  $39.6-43\ \mu$ ; lat. anth.,  $9.9-12.5\ \mu$ ; long. anth.,  $5-7\ \mu$ .

*Habitat*.—Along with *Oe. areolatum* Lagerheim, forma *ellipsosporum* form. nov., Kunda Ghat, Gorakhpur; October 7, 1937.

The alga may be compared with *Oe. areolatum* Lagerheim on account of the areolate middle layer of the spore-wall, superior pore; but, it differs from the same in obovoid to ellipsoid oogonia, obovoid to ellipsoid oospores, 2-5 antheridia and much smaller dimensions of all parts. It further contrasts with the above species in possessing dentate ribs in the middle layer of the spore-wall.

18. *Oedogonium howardii* West Tiffany, *op. cit.*, 1930, p. 101, Pl. XXXIII, Fig. 293.

forma *tenuis* form. nov. (Fig. 8, C-D).

Dioecious, macrandrous; oogonium intercalary, occurring singly or in pairs, globose or sub-globose; operculate, division median, wide and distinct. Oospores globose, filling oogonia; spore-wall smooth. Suffultory cells unswollen; vegetative cells slightly capitellate. Antheridia in rows of 8-12; sperms 2, divisions horizontal.

Lat. cell. veg. fem.,  $7-8.5\ \mu$ ; long. cell. veg. fem.,  $16.5-20\ \mu$ ; lat. cell. veg. masc.,  $9-11\ \mu$ ; long. cell. veg. masc.,  $49-53\ \mu$ ; crass. oog.,  $23-26.4\ \mu$ ; long. oog.,  $23-26.4\ \mu$ ; crass. oosp.,  $20-23\ \mu$ ; long. oosp.,  $20-23\ \mu$ ; lat. anth.,  $9-10\ \mu$ ; long. anth.,  $9-10\ \mu$ .

*Habitat*.—Along with *Oe. geniculatum* Hirn. var. *indicum* var. nov. Gorakhpur; November 7, 1937.

The form agrees with the type in globose or sub-globose oogonia, operculate median division, and globose oospore with smooth spore-walls; but, it differs from the same in possessing slightly capitellate cells and smaller dimensions of all parts.

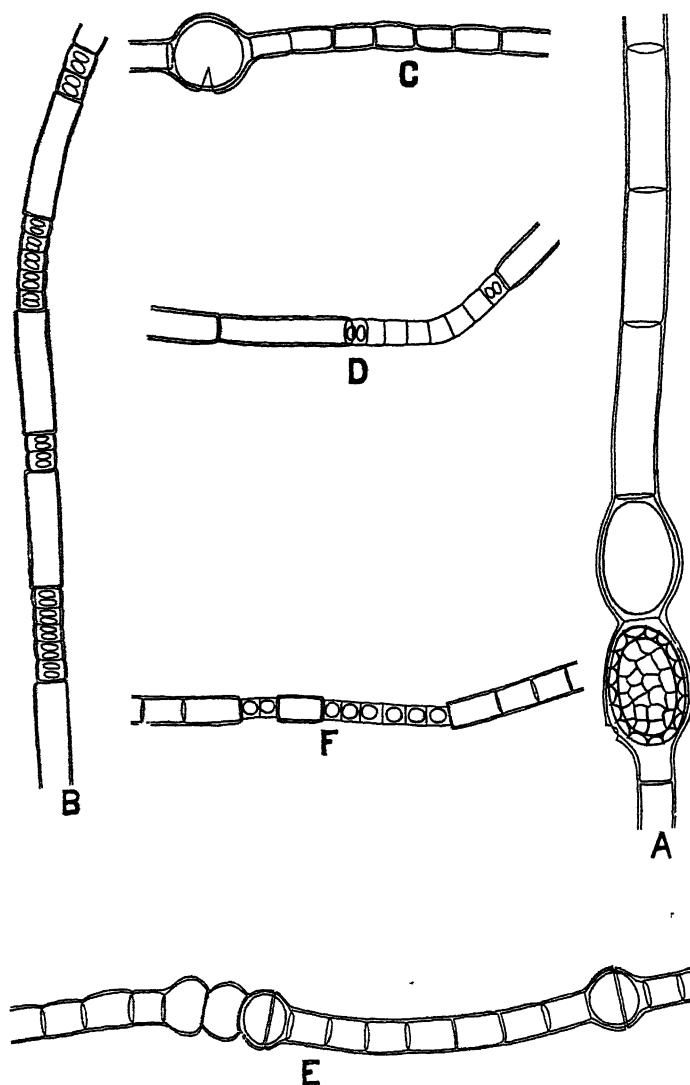


FIG. 8.

A.—A female filament with oogonia and oospores, and B.—a portion of filament with antheridia of *Oe. mesodentatum* sp. nov.; C.—a female filament with oogonium and oospore, and D.—a portion of filament with antheridia of *Oe. Hawardii* West forma *tenuis* form. nov.; E.—a female filament with oogonia and oospores, and F.—a portion of filament with antheridia of *Oe. mitratum* Wittrock var. *minor* var. nov. All  $\times 346$ .

19. *Oedogonium mitratum* Wittrock Hirn, *op. cit.*, 1900, p. 302, Pl. XXIV, Fig. 132; Tiffany, *op. cit.*, 1930, p. 105, Pl. XXXVI, Figs. 334 and 335.

var. *minor* var. nov. (Fig. 8, E-F).

Dioecious, macrandrous; oogonia intercalary, occurring singly or in pairs or in threes, depressed-globose to pyriform; operculate, division supra-median or nearly superior, narrow but distinct. Oospores of the same shape as oogonia, filling them; spore-walls thin and smooth. Suffultory cells unswollen; vegetative cells slightly capitellate. Antheridia in rows of 2-6; sperm 1.

Lat. cell. veg. fem., 9-12  $\mu$ ; long. cell. veg. fem., 17-24  $\mu$ ; lat. cell. veg. masc., 6.6-7.5  $\mu$ ; long. cell. veg. masc., 14-18  $\mu$ ; crass. oog., 20-23  $\mu$ ; long. oog., 17-20  $\mu$ ; crass. oosp., 20-22  $\mu$ ; long. oosp., 17-19  $\mu$ ; lat. anth., 6.6-7  $\mu$ ; long. anth., 6.6-7  $\mu$ .

*Habitat*.—Gorakhpur; October 19, 1937.

The variety resembles the type in 1-3 depressed-globose oogonia with a supra-median or superior division, oospores with smooth spore-walls and unswollen suffultory cells; but, it differs from the same in pyriform oogonia and oospores, single sperm and much smaller male vegetative cells in comparison to the female ones. It further contrasts with the type in smaller oogonia and oospores.

20. *Oedogonium kushmiense*, sp. nov. (Fig. 9, A-D).

Dioecious, macrandrous, oogonia intercalary, occurring singly, obpyriform to oblong-ellipsoid; poriferous, pore superior. Oospores obovoid to globose-ellipsoid, not filling oogonia lengthwise, inflating lower part of oogonia; spore-walls thin and smooth. Suffultory cells unswollen or sometimes slightly swollen; apical cell obtuse; vegetative cells cylindrical. Antheridia in rows of 2-4; sperms 2, divisions vertical.

Lat. cell. veg. fem., 16.5-18  $\mu$ ; long. cell. veg. fem., 36.3-42  $\mu$ ; lat. cell. veg. masc., 14-15  $\mu$ ; long. cell. veg. masc., 29.7-39.6  $\mu$ ; crass. oog., 42-46  $\mu$ ; long. oog., 59.4-65  $\mu$ ; crass. oosp., 36-39.6  $\mu$ ; long. oosp., 42-46.2  $\mu$ ; crass. anth., 13.2-15  $\mu$ ; long. anth., 5-6.6  $\mu$ .

*Habitat*.—Kushmi Forest, Gorakhpur; October 16, 1937.

The alga may be compared with *Oedogonium langeniforme* Hirn on account of its obpyriform and inferiorly inflated oogonia and superior pore;

but, it differs from the same in bigger dimensions of all the cells. It further contrasts with the above species in its obovoid oospores which sometimes are filling oogonia, obtuse apical cells and vertical divisions of the sperms.

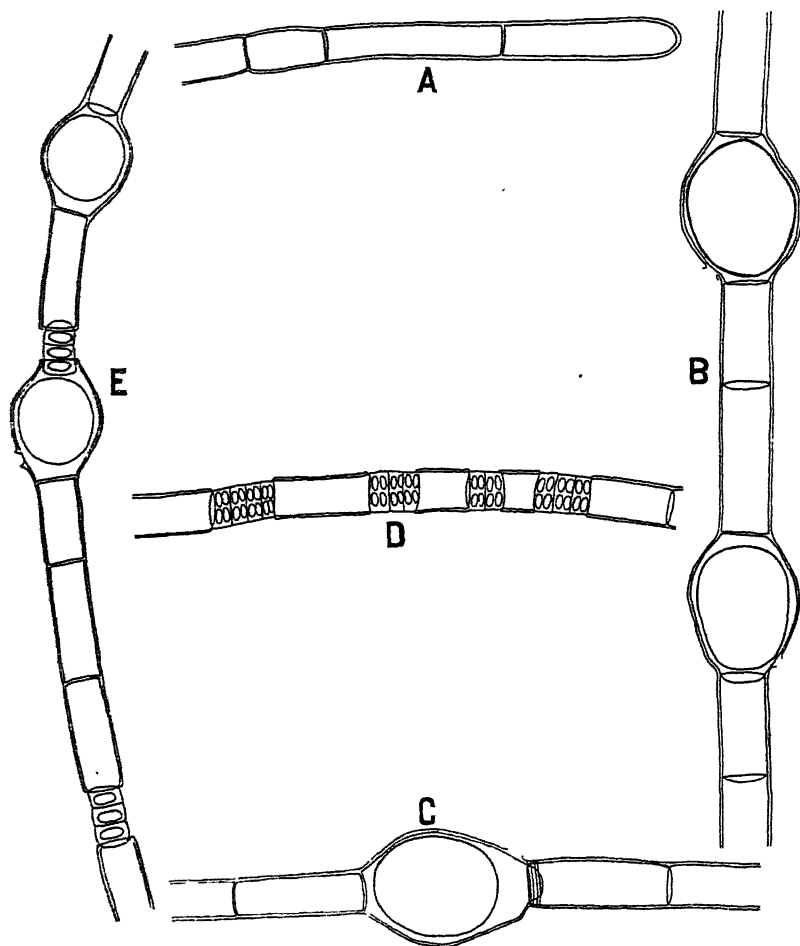


FIG. 9.

A.—A portion of filament showing apical cell, B-C.—female filaments with oogonia and oospores, and D.—a portion of filament with antheridia of *Oe. kushmiense* sp. nov.; E.—a filament of *Oe. intermedium* Wittrock forma *tenuis* form. nov. showing oogonia, oospores and antheridia. All  $\times 346$ .

C. Section *Monoica macrandria*.—

21. *Oedogonium intermedium* Wittrock Hirn, *op. cit.*, 1900, p. 94, Pl. V, Fig. 31; Tiffany, *op. cit.*, 1930, p. 72, Pl. XIV, Fig. 134.

forma *tenuis* form. nov. (Fig. 9, E).

Monoecious, macrandrous; oogonia single, intercalary, globose to depressed-globose; poriferous, pore superior. Oospores globose, filling oogonia; spore-walls thin and smooth. Antheridia in rows of 2-4, sub-epigynous or hypogynous; sperm 1.

Lat. cell.  $14.2-16.5\ \mu$ ; long. cell.,  $30-37\ \mu$ ; crass. oog.,  $26.4-30\ \mu$ ; long. oog.,  $30-34\ \mu$ ; crass. oosp.,  $26.4-27\ \mu$ ; long. oosp.,  $26.4-27\ \mu$ ; lat. anth.,  $13.2-14.5\ \mu$ ; long. anth.  $6.6-13\ \mu$ .

*Habitat*.—Along with *Oe. lautummarum* Wittrock forma *tenuis* form. nov., Gorakhpur; October 15, 1937.

The form differs from the type in possessing smaller dimensions of all parts and also in having only one sperm.

22. *Oedogonium hirnii* Gutwinski Hirn, *op. cit.*, 1900, p. 93, Pl. V, Fig. 29; Tiffany, *op. cit.*, 1930, p. 73, Pl. XIV, Figs. 136 and 137.

*Forma* (Fig. 10, A).

Lat. cell.,  $9.9-13.2\ \mu$ ; long. cell.,  $33.3-56\ \mu$ ; crass. oog.,  $33-34\ \mu$ ; long. oog.,  $33.3-40\ \mu$ ; crass. oosp.,  $26.4-28\ \mu$ ; long. oosp.,  $26.4-28\ \mu$ ; lat. anth.,  $9.9-10.5\ \mu$ ; long. anth.,  $3.3-4.2\ \mu$ .

*Habitat*.—Gorakhpur; November 5, 1937.

The form differs from the type in possessing smaller oospores and antheridia.

23. *Oedogonium geniculatum* Hirn Hirn, *op. cit.*, 1900, p. 106, Pl. VIII, Fig. 48; Tiffany, *op. cit.*, 1930, p. 78, Pl. XVI, Fig. 155.

var. *indicum* var. nov. (Fig. 10, B).

Monoecious, macrandrous; oogonia single or rarely in pairs, intercalary, obovoid or obovoid-globose; poriferous, pore median. Oospores globose, filling oogonia; spore-walls thick and smooth. Suffultory cells slightly swollen; apical cell apiculate; basal cell elongate. Antheridia in rows of 1-4, sub-epigynous or sub-hypogynous; sperms 2, divisions horizontal.

Lat. cell.,  $29.7-33\ \mu$ ; long. cell.,  $59.4-99\ \mu$ ; crass. cell. suffult.,  $33-35\ \mu$ ; crass. oog.,  $42.9-46\ \mu$ ; long. oog.,  $49.5-52\ \mu$ ; crass. oosp.,  $42.9-45\ \mu$ ; long. oosp.,  $42.9-45\ \mu$ ; lat. anth.,  $29.7-31\ \mu$ ; long. anth.,  $6.6-7.5\ \mu$ .

*Habitat*.—Gorakhpur; November 5, 1937.

The variety differs from the type in oospores completely filling oogonia, median pore and in possessing smaller dimensions of all parts.

24. *Oedogonium tapeinosporum* Wittrock Hirn, *op. cit.*, 1900, p. 297, Pl. XXIII, Fig. 117; Tiffany, *op. cit.*, 1930, p. 159, Pl. XXXIV, Fig. 314.

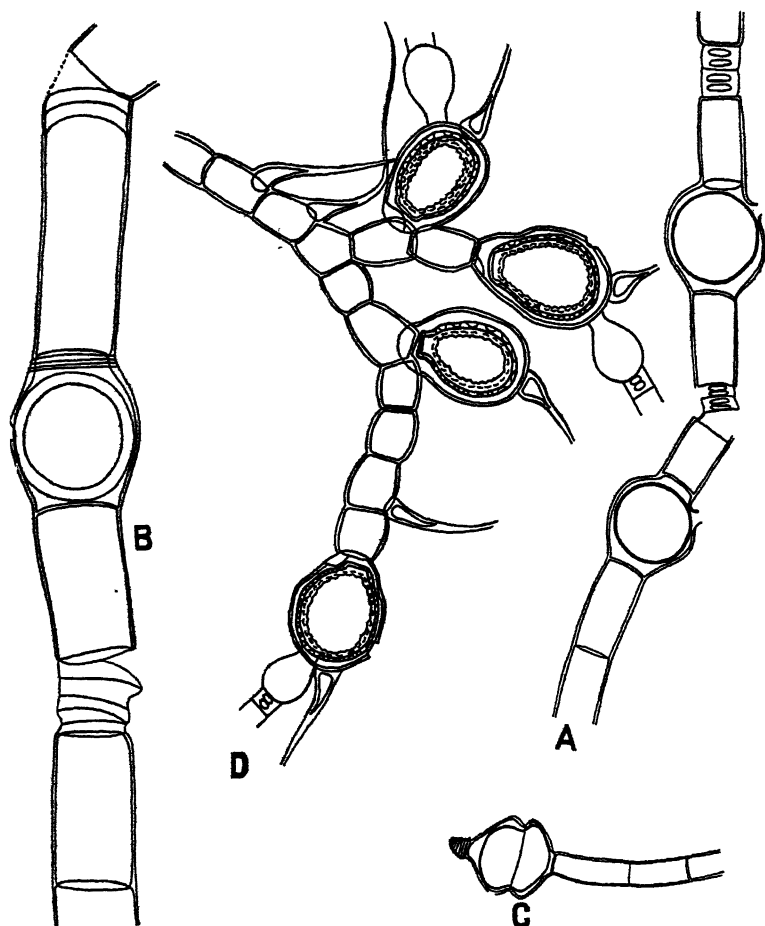


FIG. 10.

A.—A filament with oogonia, oospores and antheridia of *Oe. hirnii* Gutwinski *Forma*; B.—a filament with oogonium, oospore and antheridia of *Oe. geniculatum* Hirn var. *indicum* var. nov.; C.—a filament with oogonium and oospore of *Oe. tapeinosporum* Wittrock *forma minor* form. nov.; and D.—*Bulbochaete Bharadwajai* sp. nov.

A—B  $\times 346$ ; C  $\times 733$ ; D  $\times 346$ .

*forma minor* form. nov. (Fig. 10, C).

Lat. cell.,  $3.3-4\ \mu$ ; long. cell.,  $13.2-17\ \mu$ ; crass. oog.,  $12-13\ \mu$ ; long. oog.,  $12-14\ \mu$ ; crass. oosp.,  $10-12\ \mu$ ; long. oosp.,  $11-13\ \mu$ .

*Habitat*.—Along with *Oedogonium longatum* Kuetzing *Forma*, Benares; November 15, 1937.

The form differs from the type in possessing smaller oogonia and oospores which are completely filling the former.

Genus *Bulbochæte* Agardh.

25. *Bulbochæte Bharadwajai* sp. nov. (Fig. 10, D).

Dioecious, nannandrous, idioandrosporous; oogonia obovoid to sub-cylindric-ovoid, patent or erect, below terminal setæ; poriferous, pore superior; division of suffultory cell superior or nearly supreme. Oospores obovoid to sub-cylindric-ovoid; spore-walls thick, 3-layered, finely scrobiculate; androsporangia 3-5 seriate; nannandria near or on oogonia or scattered on vegetative cells; antheridia exterior, 3-4.

Lat. cell., 12-16.5  $\mu$ ; long. cell., 16.5-19.8  $\mu$ ; crass. oog., 26.4-29.7  $\mu$ ; long. oog., 38-42.9  $\mu$ ; crass. oosp., 23.1-26.4  $\mu$ ; long. oosp., 33-36.3  $\mu$ ; lat. androsp., 12-13.2  $\mu$ ; long. androsp., 12.5-14.5  $\mu$ ; lat. nannand. stipes., 11.5-13.2  $\mu$ ; long. nannand. stipes., 16.5-19.8  $\mu$ ; lat. anth., 6.6-8.3  $\mu$ ; long. anth., 4.5-5  $\mu$ .

*Habitat*.—Floating on water, along with *Zygnema gorakhporensis* sp. nov., Gorakhpur; October 8, 1936; November 5, 1937.

The alga can only be compared with *Bulbochæte borealis* Wittrock on account of its dioecious nannandrous habit and scrobiculate spore-wall; but, it differs from the same in its idioandrosporous habit, obovoid to sub-cylindric-ovoid erect oogonia, oospores of the same shape as the oogonia, thick 3-layered spore-walls, external antheridia and straight and bigger stipes of the nannandria. It further contrasts with the above species in its smaller cells, narrower and longer oogonia and oospores and bigger dwarf males.

The writer takes this opportunity to express his great indebtedness to Professor Y. Bhâradvâja, for his kind guidance and criticism throughout the course of this investigation, and also to Professor S. L. Ghose, for many helpful suggestions.

# THE OCCURRENCE AND INHERITANCE OF PURPLE-TIPPED GRAINS IN SORGHUM.

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THE sorghum grain is naked and develops outside the glumes which clip the base of it. Besides the brownish scar of the desiccated stylar base, the rest of the grain is usually of a smooth whole colour. One other interference with the wholeness in colour is from the waterlines that divide the grain surface into segments.

In some of the varieties of Kafir (*Sorghum caffrorum*, Beauv.) imported from America, there is a prominent purple spot at the tip of the grain round the remnants of the stylar base (see illustration). This spot varies from one to half a millimeter in diameter. It is more oval than round, the length of the oval being along the suture lines that run on either side of the stylar fork. This spot develops colour at the dough stage of the grain when the seed coat begins to harden up. It shows best when the grain is dry. The spot is seen prominently in some of the Kafirs and is used for varietal distinction in America (Vinall, *et al.*, 1936). This purple-tip is thus a characteristic of some Kafir varieties which are American in immediate origin. An examination of Kafirs that were got down directly from Africa does not show this spot in clearness. In some varieties there is a dull spot in this region suggestive of potentiality of colour. It looks as if this potentiality has been activated under the environmental conditions of America where it was introduced from Africa. American purple-tipped varieties grown at Coimbatore have retained the tip colour undiminished after four seasons of growth.

The colour belongs to the same chain of colour manifestation as that represented by the leaf-sheath-glume series. Wherever the gene 'Q' (Rangaswami Ayyangar, *et al.*, 1933), is present, it is reddish purple and in the presence of 'q' it is blackish purple. The colour is confined to the pericarp layer being concentrated at the cuticle epicarp and hypoderm at the top and in the cross and tube cells at the bottom, with a rarified distribution in between. The oval purple patch is definite and concentrated, though the adjacent tissues are tinged with a lighter wash of the pigment. This

wash is noticeable in the adjacent grain suture grooves. The pericarp colour in the grain develops in wholeness when the 'W' factor is present (Rangaswami Ayyangar, *et al.*, 1933). When it is absent the colour is confined to the base of the grain under the protection of the glumes. This new purple-tipped grain character is independent of 'W' factor determining pericarp colour manifestation in wholeness. This purple tip is therefore also present in Kafir varieties, with a pink grain (the 'yellow' of the Americans). This character is absent in other varieties from America and in all Asiatic and Chinese varieties so far examined at Coimbatore. The gene responsible for this purple spotting at the tip of the grain seems to be present in some varieties of Kafir, African in origin. In Africa, the manifestation of this character is feeble; in America, it is patent.

The American variety Kalo, is a resultant of a cross between Kafir and Milo (Swanson and Lande, 1934). Kalo is pink in colour and has a purple-tipped grain. Of the purple-tipped varieties examined so far Kalo shows this tip purple most prominently.

In the Kalo variety imported from Colorado in 1935, among a majority of purple-tipped plants there was a minority population with no purple-tipped grain. Three of these were taken and sown. In one of these there appeared a number of purple-tipped plants suggestive of taint from pollen from similar plants. In a further generation one of these purple-tipped grain natural crosses gave rise to 109 plants with purple tip and 36 with no purple-tip on the grain. From this  $F_2$ , a third generation was raised and the behaviour of the selections is given below.

Family No.	Character of Selection	Progeny Behaviour Grain Tip		
		Purple	No Purple	
A.S. 6142	Grain tip No Purple ..	..	Pure	
„ 6143	„ „ ..	..	Pure	
„ 6138	„ Purple ..	123	44	
„ 6139	„ „ ..	127	39	
„ 6140	„ „ ..	146	44	
„ 6141	„ „ ..	131	45	
	TOTAL ..	527	172	
	Expected 3 : 1 ratio ..	524.25	174.75	

$$\chi^2 = .023 \quad P > 0.8.$$

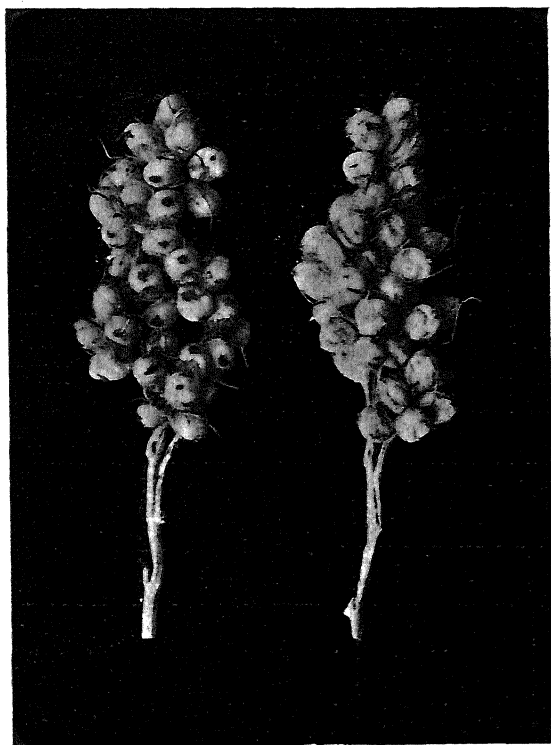
From the above table it will be seen that the purple tip on the grain is a simple dominant to its absence. A gene designated  $P_{GT}$  gives rise to a purple spot round the stylar scar at the tip of the grain. The common grain sorghums without a purple-tip are  $p_g$  in genic constitution. The disappearance of this dominant purple character from most of the other cultivated varieties of grain sorghum throws light on the origin and spread of cultivated sorghums.

#### Summary.

Almost all grain sorghums are without a purple spot on the top of their grains. In these, the tip of the grain has merely the brownish scar left by the dried stylar base. In some varieties of Kafir the stylar scar is surrounded by a small purple spot. In African Kafirs this purple spot is either feeble or latent. In some Kafirs from America the spot is patent. In crosses with varieties with no purple-tip on the grain the purple tipped grain behaves as a simple Mendelian dominant to the common tipless condition. The gene responsible for this purple-tip on the top of the grain has been designated  $P_{GT}$ . In the presence of 'Q' factor this spot is reddish purple. With 'q' it is blackish purple. The purple on the tip of the grain belongs to the same series as the leaf-sheath purple, and the glume purple that goes with it. This factor is independent of the factor 'W' which determines the manifestation of pericarp colour in wholeness. The presence of this dominant colour character in the African Kafir and its disappearance in other cultivated sorghum throws light on the origin and distribution of many of the cultivated sorghums.

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Purple-tipped Ordinary Sorghum grains.



# THE NATURAL ACTIVATORS OF PAPAIN.

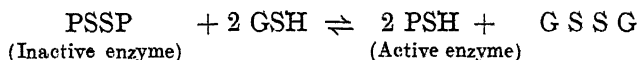
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Received November 10, 1938.

THE identification of the "kinases" of liver<sup>1</sup> and yeast<sup>2</sup> proteinases with glutathione has aroused considerable interest in the study of natural activators of the analogous plant enzyme, papain. Maschmann<sup>3</sup> reported that the "begleitstoff X" of papain was a sulphur containing substance, *not* identical with SS-glutathione but an unidentified disulphide containing polypeptide. Grassmann<sup>4</sup> isolated the substance from commercial papain and found that it was a peptide containing cysteine and glutamic acid but was *not* glutathione.¶

From a study of the influence of oxidising and reducing agents on the activity of papain, Bersin<sup>7</sup> brought evidence to show that "the active enzyme is to be looked upon, as a thiol compound while its inactive form is a disulphide". Purr<sup>8</sup> concluded from his experiments on the reversible oxidation of papain that the enzyme is probably an SH-protein. The reversibly inactivated SS-form was capable of being activated by SH-compounds like cysteine, glutathione and SH-proteins, the activation of the enzyme being represented thus :—



SH-compounds co-existing with the enzyme in the plant, may therefore be considered as natural activators. Grassmann's work had definitely excluded glutathione from this group.

A large part of the previous work refers to commercial papain. The susceptibility of the SH-compounds to oxidation and decomposition|| during

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¶ More recently Frankel and Maimin<sup>5</sup> reported the separation of the natural activator from the latex of *Carica papaya* and pointed out that "the chemical behaviour of the natural activator and of glutathione on the hydrolysis of peptone and gelatin make it probable that the natural activator is glutathione. A decisive proof on this question must await complete chemical analysis." This note appeared at about the same time as our preliminary contribution on the subject.<sup>6</sup>

|| We have observed the production of hydrogen sulphide during the storage of papaya latex, under toluene, in a refrigerator.

the manufacture and storage of papain naturally renders results of any work on natural activators from commercial preparations, somewhat uncertain. A reinvestigation of the problem using fresh latex from the fruit of *Carica papaya* appeared justified. It was relevant also to examine the natural activators of other papainases from pine apple (*Bromelia sativa*) and cucumber (*Cucumis sativus*). The present investigation is concerned with a qualitative identification of some of the SH-compounds in plant juices which are reputed sources of proteinases and their quantitative estimation.

### *Experimental.*

*Estimation of the total SH-compounds.*—(1) The iodimetric titration was employed for the purpose.<sup>9</sup> A correction for the accompanying ascorbic acid was applied by estimating the vitamin by the micro-method of Birch *et al.*<sup>10</sup>

5 grams of the freshly tapped latex from the papaya fruit were rubbed in a glass mortar with washed sand and 20 per cent. trichloroacetic acid and filtered on a Buchner. The residue was repeatedly extracted with the acid till the filtrate gave no nitroprusside test. The combined filtrates were made up to 100 c.c. with water, the final concentration of the acid in the filtrate being adjusted to 10 per cent. 2 c.c. aliquots were employed for each titration.

(2) The photoelectric colorimeter was also employed for the estimation of the total SH-compounds. The intensity of the colour produced in the nitroprusside reaction is a measure of the SH-content. The arrangement was identical with the one we have used for studying the kinetics of succinic dehydrogenase.<sup>11</sup> The cell contained 10 c.c. of the solution made up as follows: 2 c.c. of saturated ammonium sulphate, 1 c.c. of 2 per cent. sodium nitroprusside, 6 c.c. of latex extract (after treatment with trichloroacetic acid). When the cell was placed in position, 1 c.c. of liquor ammonia was added, quickly stirred with the pipette itself, and the ammeter reading taken. As the colour fades rapidly, it is essential that the reading should be taken immediately after adding ammonia. The concentration of SH is obtained by referring to a standard curve showing the relation between ammeter reading and concentration of glutathione. This method eliminates the need for a parallel estimation of ascorbic acid, so essential in the iodimetric method.

*Estimation of Glutathione.*—The manometric method of Woodward<sup>12</sup> based on the specific activation of glyoxalase by reduced glutathione was employed.

This method is of particular value in distinguishing this tripeptide from other associated SH-compounds in plant juices.

As a source of glyoxalase, both acetone yeast and liver extract were employed. The acetone yeast however soon became inactivated and so was abandoned in favour of the liver preparation. The press juice of pig's liver was dialysed against distilled water in collodion bags in a refrigerator until free from SH-compounds (12 hours' dialysis was found adequate). The dialysed solution mixed with an equal volume of glycerine, gives a preparation which kept in the ice-box retains its activity for at least a month. Methyl glyoxal was obtained by distilling dihydroxy acetone, prepared from glycerine by the Neuberg's method<sup>13</sup> over 10 per cent. sulphuric acid, using aluminium sulphate as catalyst.

The procedure for the estimation of glutathione was the same as that of Woodward. A reference curve was first obtained connecting changes in the manometric reading with increasing concentration of glutathione—0.025, 0.05, 0.10, 0.15 and 0.20 mg. The total volume of the reaction mixture was 2.00 c.c. made up of 0.5 c.c. methyl glyoxal, 0.5 c.c. glyoxalase, 0.2 c.c. (0.5 M.) sodium bicarbonate, and 0.8 c.c. of neutralised extract of fresh latex. The temperature of the thermostat was maintained at 28° 0 C. ( $\pm 0.02$ ). Sulphosalicylic acid was used as the protein precipitant. 2.5 g. of the fresh latex were rubbed with 1 c.c. of 1 M. sulphosalicylic acid and made up to 25 c.c.; aliquots of the clear filtrate, neutralised to methyl red with 0.5 M. sodium bicarbonate, were used for the glutathione estimation. In later experiments, sodium metaphosphate<sup>14</sup> was employed as protein precipitant with very satisfactory results.

The glutathione contents of some plant juices are given in Table I.

TABLE I.

Material				Total SH-compounds calculated as glutathione mg. per 100 g. of material	Glutathione mg. per 100 g. of material	REMARKS
<i>PAPAYA (Carica papaya)</i>						
Latex from fruit	Sp. 1	..	..	2460	120	
"	" 2	..	..	1470	70	
"	" 3	..	..	1560	110	
"	" 4	..	..	..	122	Free Cysteine absent
"	" 5	..	..	..	302	
"	" 6	..	..	2456	221	
"	" 7	..	..	2156	124	
"	" 8	..	..	..	73	
"	" 9	..	..	2750, 2750*	..	
"	" 10	..	..	2887, 2800*	..	
"	" 11	..	..	3288	..	
"	" 12	..	..	2148, 2100*	..	*Photocolori- metric method.
"	" 13	..	..	3308, 3260*	..	
<i>Cucumber (Cucumis sativus).</i>						
Ripe fruit : press juice from rind	..	..	..	..	57.5	
"	" pulp	..	..	..	traces	
"	" whole fruit	..	..	80	50	
Papaya latex : Green unripe fruit (Sp. 14)	..	..	..	..	88	The latexes were obtained from the same tree on the same day.
" Ripe fruit (Sp. 15)	..	..	..	..	340	
Papaya leaf latex (Sp. 16)	..	..	..	..	65	
<i>Calatropis gigantea</i>	..	..	..	174	traces	
<i>Ficus bengalensis</i>	..	..	..	..	34	
Pine apple ( <i>Bromelia sativa</i> )	..	..	..	28	Present	

*Preparation of Glutathione from Papaya latex.*—Pirie's method<sup>15</sup> was employed for the purpose. Starting with 100 grams of the latex the characteristic silky white, crystalline precipitate of cuprous glutathione was obtained. The isolation of glutathione itself was not possible owing to the scarcity of the raw material. On decomposing an aqueous suspension of the cuprous compound with  $H_2S$  and then removing the  $H_2S$  by bubbling a stream of hydrogen, a filtrate which gave intense nitroprusside test, was obtained.

Cysteine gives with dimethyl-*p*-phenylene diamine and ferric chloride a blue colour. The test is specific to cysteine.<sup>16</sup> Fresh latex of *Carica papaya* does not contain free cysteine.

#### Discussion.

The results given in Table I show that glutathione is a constituent of the latex of *Carica papaya* to the extent of 5 to 10 per cent. of the total SH-compounds. It has been observed that on storing, there is a fall in the glutathione content. The occurrence of glutathione in the latex, admixed with large concentration of SH-compounds coupled with the fact that its concentration falls during storage, probably accounts for the failure of earlier workers to detect and isolate glutathione in the latex.

The glutathione content of the latex derived from the ripe fruit is much higher than that from a green and unripe fruit obtained from the same tree. The latex obtained from snapping a leaf near the trunk has a much lower glutathione concentration. The yield of the latex from the ripe fruit is very much lower than that from a green one.

In the case of cucumber, the glutathione is confined to the outer regions of the ripe fruit, the inner pulp containing little or no glutathione. On scratching the outer rind, a gummy exudate is obtained which gives a powerful nitroprusside test. There are definite regions near the periphery of the fruit to which the presence of glutathione is confined. These can be detected by immersing a section of the fruit in ammonium sulphate and sodium nitroprusside and then exposing to ammonia. An evanescent pink colour reveals the regions of glutathione.

The press juices of pine apple, *Ficus bengalensis* and *Calatropis gigantea* also contain glutathione. The peptide is thus a natural constituent of all plant juices which are reputed sources of proteinases. The natural activator of 'papainases' is thus identical with that of liver cathepsin or yeast proteinase.

It is not however possible to say, at this stage, that glutathione is the only natural activator of papain. Grassmann has already isolated a cysteine-

containing dipeptide from commercial papain. The observation that the glutathione content falls off during storage makes it probable that the dipeptide is derived from glutathione by enzymic hydrolysis. Before venturing on a definite statement, it is necessary to prepare a complete balance sheet of the sulphhydryl compounds present in the latex.

### Summary.

The presence of glutathione in the latex of *Carica papaya* and the press juices of pine apple, cucumber, and *Ficus bengalensis*, has been established.

The tripeptide has been isolated from the latex of *Carica papaya* in the form of its cuprous compound.

A quantitative estimation of glutathione has been made, employing the specific glyoxalase reaction.

Glutathione is shown to be the natural activator of plant proteinases which in this respect also, are analogous to liver cathepsin and yeast proteinase.

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# THE SUBCUTANEOUS CORPORA ADIPOSA IN *RANA TIGRINA* DAUD.\*

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(Communicated by Mr. Beni Charan Mahendra, F.Z.S.)

## *Introduction.*

THE presence of fatty deposits below the skin is rather a rare phenomenon in Amphibians, and only very few genera have so far been recorded as possessing them. *Proteus* (Maurer, 1911) has a thin sheet of such fatty deposit. *Bufo*, *Hemisus* and *Uperodon* (= *Cacopus*) have more definite, localised regions of such tissue. In *Bufo*, Leydig (1887) noted the presence of fat-bodies in the axillary and inguinal regions, and observed that they are reddish-yellow in *B. calamita* and greyish-yellow in *B. variabilis*. Later, Boulenger (1910) examined 20 species of this genus and reported the presence, in 15 of them, of a pair of subcutaneous fat-bodies at the junction of the hind-limbs with the trunk. He did not find such bodies in a number of other Anura, including European representatives of the families *Hylidae*, *Pelobatidae* and *Discoglossidae*. In the case of *Hemisus*, Cope (1889) remarked on the presence of subtriangular external corpora adiposa near the shoulder-girdle, between the external and internal oblique muscles; and in the same genus, Beddard (1908) observed "three pairs of large-lobed fat-bodies, of which one pair correspond in position to the thymus in other Frogs, the second lie in a cavity (? a lymph-sac) behind the shoulder girdle, and the third pair are contained in a sac partly overlapping the thigh, which is to be compared with the saccus iliacus of *Rana*." In *Uperodon* (= *Cacopus*), Devanesen (1922) found subcutaneous fat deposits in two situations: in the cervical region, and in the inguinal. The former he called "the cervical fat-body", and the latter "the supra-inguinal".

Amongst the more recent writers on Amphibia, Werner (1931) and Versluys (1931) make no mention of any subcutaneous fat-bodies, while Noble (1931) speaks of the occurrence of a conspicuous fat-body just anterior to the clavicles in some frogs (he, however, does not specify the species!), and adds that various narrow-mouthed toads have such structures under the skin.

As far as the genus *Rana* or the family *Ranidae* is concerned, apart from a brief passing remark of Devanesen (1922), who reports on the occasional

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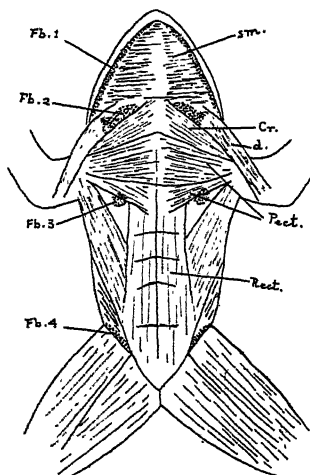
\* Work done at the Department of Zoology, St. John's College, Agra.

presence of subcutaneous fat in the neck region in *Rana hexadactyla* and *R. breviceps*, there is apparently no previous record of the occurrence of fat-bodies under the skin. Ecker (1889) does not observe them, nor does any of the prevalent text-book writers (Marshall, 1914; Whitehouse and Grove, 1933; Holmes, 1930, etc.). Gaupp (1899) says, "Eigentliche Lymphdrüsen besitzt der Frosch nicht. Doch sind ausser der Thymus noch einige besondere 'lymphadenoid' Organe vorhanden." Whether these 'lymphadenoid' organs of Gaupp correspond to subcutaneous fat-bodies, it is hardly possible to say, as Gaupp does not give further details about them. He, however, does not mention the presence of adipose tissue in this connection.

*General Account.*

In *Rana tigrina*, the subcutaneous fat-bodies (*Corpora Adiposa*) occur in five pairs below the skin, four of which are on the ventral surface and only one is on the dorsal. Besides these definitely localised regions of adipose tissue, fat may occur in irregular patches in various other parts as well. Especially is it to be found on the inner aspect of the dorsal skin, in the depressions due to the warts and longitudinal folds; as also here and there in connection with the lymphatic septa.

(1) *Submandibular Fat-bodies* (Text-Fig. 1, *Fb. 1*).—Fatty deposit occurs in the whole of *Musculus submaxillaris* (Gaupp)<sup>1</sup> and gives a darkish-



TEXT-FIG. 1. Ventral view of *Rana tigrina* after the removal of skin, showing the situation of subcutaneous fat-bodies.

Cr.—*M. coraco-radialis*; d.—*M. deltoideus*; Fb. 1.—Submandibular fat-body; Fb. 2.—Pre-pectoral fat-body; Fb. 3.—Axillary fat-body; Fb. 4.—Inguinal fat-body; Pect.—*M. pectoralis*; Rect.—*M. rectus abdominis*.

<sup>1</sup> In the present paper all muscles are named after Gaupp.

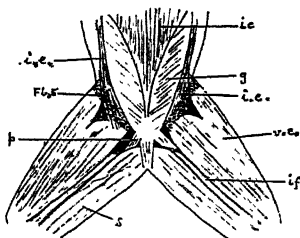
yellow<sup>2</sup> appearance to this muscle. Teased preparations examined under the microscope prove the presence of fat beyond doubt. This fatty deposit is better developed all along the edge of the dentary at the line of origin of *M. submaxillaris* (i.e., along the line of the "Pars affixa" of the skin to the head). In some instances, the deposit extends as a fairly conspicuous strip of adipose tissue all along the inner edge of the lower jaw from a little behind the junction of the mandibular rami to the angle of the jaws.

(2) *Pre-pectoral Fat-bodies* (Text-Fig. 1, *Fb.* 2).—The second pair of ventral subcutaneous fat-bodies occurs just behind the vocal sacs in the male, and in the position corresponding to it in the female. It is located on the anterior aspect of *M. coraco-radialis* and is continued over even on the skin of this region. It is developed in connection the lymphatic *septum submaxillaris*, and narrow strips from it extend behind or over the angle of the jaw and overlie the *M. depressor mandibulæ* behind the ear-drum.

(3) *Axillary Fat-bodies* (Text-Fig. 1, *Fb.* 3).—The third pair of subcutaneous fat-bodies occurs on the ventral side of the trunk behind the axillary region. When the skin is removed from this area, it is seen through the muscles as an indistinct yellow patch, covered over by the *Portio abdominalis* of the *M. pectoralis*. In some specimens this pair is well developed and extends laterally up to the *M. coraco-brachialis*.

(4) *Inguinal Fat-bodies* (Text-Fig. 1, *Fb.* 4).—The last ventral subcutaneous fat-body is developed on the *septum inguinalis*, and sends a narrow piece laterally to join the dorsal fat-body in this region, so that the two together lie like a half-loop on the anterior aspect of the root of the thigh.

(5) *Dorsal Fat-bodies* (Text-Fig. 2, *Fb.* 5).—This pair is developed under the skin on the dorsal side of the posteriormost part of the trunk in connection



TEXT-FIG. 2. Dorsal view of posterior part of *Rana tigrina* after the removal of skin.

ic.—*M. ilio-coccygeus*; i.e.—*M. iliacus externus*; i.f.—*M. ilio-fibularis*; *Fb.* 5.—Dorsal fat-body; g.—*M. glutaeus*; p.—*M. pyriformis*; S.—*M. semimembranosus*; v.e.—*M. glutaeus magnus*.

<sup>2</sup> The darkness is really due to the presence of melanophores, the number of which varies from superabundance to real paucity from individual to individual as far as these corpora adiposa are concerned.

with the septum *glutaeale superficiale*. Each of these fat-bodies lies at the junction of the thigh with the trunk and is oblique in position. When the skin is removed, they are seen to lie across the origin of *M. iliacus externus*.

#### Variations.

These fat-bodies, though fairly constant in position, vary in size, colour and extent from one individual to another. On the whole, they are always better developed in the females than in the males. Their colour ranges from bright yellow or orange to dull slate, the difference being mainly due to the abundance or paucity of melanophores. Boulenger (1910) found that the subcutaneous fat-bodies in *Bufo* diminish in size when the animals are kept without food for a considerable time. That seems to be true also for *Rana tigrina*, as emaciated specimens show these structures little or not at all developed. This observation affords a contrast to Funke's view (1899) that the fat-body can have very little influence as somatic reserve.

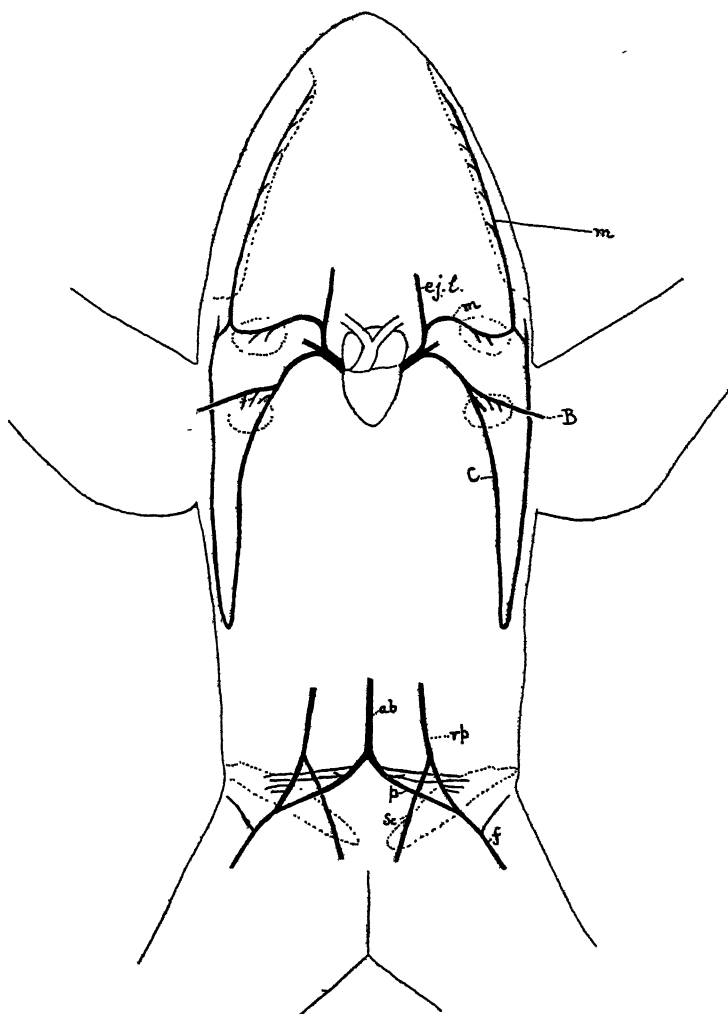
The greater or less development of the subcutaneous fat-bodies in *Rana tigrina* goes hand in hand with that of the internal, pre-renal corpora adiposa. When the latter is preponderantly developed, the subcutaneous fat-bodies also exhibit a special degree of enlargement.

#### Vascular Supply.

(a) *Veins*.—The *sub-mandibular* and the *pre-pectoral* fat-bodies (Text-Fig. 3) send minute veins to the internal mandibular vein. The *axillary* fat-bodies send veins to vena cutaneous magna. The *inguinal* fat-bodies are supplied with veins going to the pelvic, together with either a vein opening into the iliac, or with one going to the femoral at (or very near to) its bifurcation to form the iliac and the pelvic. The *dorsal* fat-bodies send, a vein each, to the femoral.

With reference to *Bufo*, Boulenger (1910) found that the inguinal fat-bodies send blood to the pelvic and the ventral abdominal veins. In *Rana*, however, I do not find any veins going from the fat-body to the abdominal.

(b) *Arteries*.—The *sub-mandibular* and the *pre-pectoral* fat-bodies receive their blood supply from a branch of the lingual; the *axial* from arteries coming from the brachial; and the *inguinal* and the *dorsal*, from a separate branch for each, coming from the iliac. The cutaneous artery also passes through the prepectoral fat-body of its side, but I have failed to discover any branches opening from it into the mass of the fat-body.



TEXT-FIG. 3. Diagram of the venous system of *Rana tigrina*, showing venous supply of subcutaneous fat-bodies.

*ab.*—Abdominal vein ; *B.*—Brachial vein ; *C.*—Musculo-cutaneous vein ; *ej.l.*—Lingual vein ; *f.*—Femoral vein ; *m.*—Mandibular vein ; *p.*—Pelvic vein ; *rp.*—Renal portal vein ; *Sc.*—Sciatic vein.

#### *Histology.*

Histologically, the subcutaneous fat-bodies have the same structure as the supra-renal corpora adiposa. I cut sections of the pre-pectoral and the inguinal ones, and both of them are made of an aggregation of large vacuolated cells, polyhedral in appearance and fatty in contents ; a mass of adenoid tissue, occupying the intercellular spaces ; and several blood capillaries cut

at various angles here and there. The whole mass is bounded by a connective tissue capsule.

### *Discussion.*

As the subcutaneous corpora adiposa may be confounded with such structures as thymus, epithelial bodies (Epithelkörperchen, Gaupp), post-branchial bodies, etc., the following discussion deals with the relevant facts in this connection.

(a) *Thymus*: Beddard (1908, pp. 915-16 and text-fig. 176 on p. 899) interprets what has been called the 'pre-pectoral fat-bodies' in this paper as the *thymus glands* in *Hemisus guttatum* and some other Anura. This view, however, is hardly tenable. The pre-pectoral fat-bodies differ from the thymus in their situation. They lie below the skin within the lymph-sac in connection with the lymphatic *septum submaxillaris*, and are situated anteriorly to *M. coraco-radialis*. They are not concealed to any extent under any muscle. The thymus, on the other hand, whenever present, lies covered over by *M. depressor mandibulæ* and can be exposed by reflecting it. According to Ecker (1889), it is "an elongated, oval body not quite 3 mm. long, lying in the space between the *M. depressor mandibulæ* and the *M. sternocleidomastoideus*; it extends slightly beyond the posterior border of the former muscle" (320). Thus it differs from the pre-pectoral fat-body even in size.

The dissection of young tailed *Rana tigrina*, 18 mm. long from snout to vent (tail 10 mm.) shows that the pre-pectoral fat-bodies and the thymus glands co-exist in the same specimen, and this fact is also opposed to Beddard's interpretation.

(b) *Postbranchial bodies, epithelial bodies, etc.*—With reference to the structure in *Hemisus* corresponding to the axillary fat-bodies of the present paper, Beddard (1908) is of opinion that they should be referred to the same category as "postbranchial bodies," "Epithelkörperchen," etc. The *epithelial bodies, parathyreoids, postbranchial bodies, suprapericardial bodies, gill remnants, ultimobranchial bodies* "arise from parts of the visceral clefts," thus being pharyngeal derivatives, "separate from their parent tissue, and, enveloped in connective tissue, sink to a deeper position." (Kingsley, 1926, p. 275.) The axillary fat-bodies appear to be too posteriorly and superficially situated to be regarded as pharyngeal derivatives and their relation to gill-clefts seems to be problematical, at least on *a priori* grounds. The question, however, can be definitely settled only by developmental studies. As I am already engaged on such investigations, I reserve my considered opinion till I have fully worked out the development of the subcutaneous corpora

adiposa. It is certain, however, that the hinder fat-bodies described herein cannot be interpreted on this basis.

### Summary.

The present paper deals with the description of subcutaneous fat-bodies found in *Rana tigrina*. It is found that apart from minor deposits of adipose tissue within the lymph spaces here and there, there are five definitely localised corpora adiposa under the skin: four on the ventral surface, and one on the dorsal. The situation of these fat-bodies in relation to muscles and lymphatic septa, their blood-supply and histology, as well as their variations in size, colour and extent are recorded.

### Acknowledgements.

My attention to the occurrence of subcutaneous fat-bodies in *Rana tigrina* was directed by Mr. Beni Charan Mahendra, under whose supervision the present work was carried out. I am much grateful to him for his constant guidance and assistance in this work. I am also indebted to the University of Agra for a research scholarship; to Canon T. D. Sully for permission to work in the Zoology Department of St. John's College, Agra; and to Prof. L. P. Mathur for the facilities enjoyed by me in the Laboratory.

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# THE DEVELOPMENT OF ANURAN KIDNEY.

## Part II. The Development of the Mesonephros of *Bufo melanostictus* Schneider.

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(Communicated by Prof. S. G. M. Ramanujam, M.A., Ph.D., D.L.C., F.Z.S.)

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### Introduction.

It has already been pointed out in my account<sup>32</sup> of the development of the mesonephros of *Rhacophorus maculatus* (Boulenger) that the great bulk of literature about Amphibian kidney is on *Rana* and that investigations are greatly needed in other genera in order to arrive at an account typical of Anura. So far there is no work devoted exclusively to the development of the mesonephros of *Bufo*. The following is an account of the formation of the mesonephros in *Bufo melanostictus* (Schneider) with a comparison of the same with that of *Rana* (Gray, 1930) and *Rhacophorus* (Reddy, 1938). Since the greater part of the development is same as in *Rana* and *Rhacophorus* only a very brief account is given excepting in places of deviations.

*Material and Method.*

The genus *Bufo* includes 85 species of toads. *Bufo melanostictus*, Schneider is the common Indian toad found in India, Ceylon and Burma, Sikhim Himalayas to a height of 10,000 feet and Nilgiris to a height of 7,000 feet. Its distribution extends also to southern China, Malay Peninsula and Archipelago. It is nocturnal in its habits. Eggs are laid in two long strings either in running water or pools. The strings are composed of gelatinous matter which swells as the tadpoles are hatched out from the eggs in about four days. For some time the tadpoles remain within the swelled gelatinous mass before they swim away.

The strings of eggs were collected and allowed to hatch in protected open-air tanks. Tadpoles at different stages were singled out and fixed in Bouin's fluid. The following stages of tadpoles were used in the course of this work :

Stage 1.—With yolk sac.

„ 2.—With external gills disappearing.

„ 3.—With external gills completely disappeared and hind legs evident.

„ 4.—With front legs developed and hind legs evident.

„ 5.—With front and hind legs completely developed.

„ 6.—With mouth widening and tail disappearing.

The stages in the development of the mesonephric units correspond to the various stages selected above with their external characters noted against them. Since the lengths of the tadpoles of the same stage showed variations it is felt not necessary to note down their stage lengths.

Fixing, dehydration, (decalcification in the case of advanced tadpoles where skull formation has begun) and embedding were done as in the case of *Rhacophorus*.<sup>22</sup> 12  $\mu$  Sections were cut and were stained in Delafield's hæmatoxylin with eosin as counter stain.

*Development of Mesonephros.*

Stages 1 and 2.—As in the case of *Rana* and *Rhacophorus* blastema occupies the dorso-median wall of the archinephric duct. The early mesonephric units arise as oval condensations of the cells of the blastema. 10 to 13 Condensations or nephroblast vesicles are found on either side of the median line. Each nephroblast vesicle is composed of 14 to 16 loosely packed cells (R.E.M.C. Fig. 1). Just in front of each nephroblast vesicle and almost in connection with it is a group of 4 to 5 cells (R.F.N. Fig. 1). This small condensation of cells appears almost at the same time as the condensation of the nephroblast vesicle. But while

the nephroblast condensation undergoes rapid reorientation and development the small condensation remains inert for a long time. This is the rudiment of the early peritoneal funnel.

As in the case of *Rana* and *Rhacophorus* the development of the nephroblast vesicles follows no regular line and the mesonephric units of the left side are better developed than those of the right. With the advent of the later malpighian units this asymmetry becomes more pronounced.

The formation of the early malpighian capsules, their glomeruli and their connected tubules is exactly like that of *Rana*. Each tubule increases in length and is initially thrown into the "Henle's loop". Subsequently, it takes to a complicated course of coiling with no definite order.

At this stage an examination of sagittal and frontal sections reveals the presence of 13 to 15 condensations of blastema cells along the dorso-median wall of the archinephric duct. These are the rudiments of the straight tubules of the later units.

*Stage 3.*—With the increase in length of the tubules of the early malpighian capsules the latter are pushed towards the peritoneal wall. At the same time the archinephric duct is also forced more and more away from the blastema. When the malpighian capsule approaches the peritoneal wall the rudiment of the early peritoneal funnel which has remained inert so far undergoes reorientation with the result that a lumen is developed within it. This is followed by the appearance of cilia in the lumen. As the malpighian capsule is pushed further towards the peritoneal wall by the growing tubule the peritoneal funnel which is wedged in between the peritoneal wall and the malpighian capsule forces a connection with the coelom. The other end of the funnel opens into a blood vessel as in the case of the previous forms (Figs. 1 to 6).

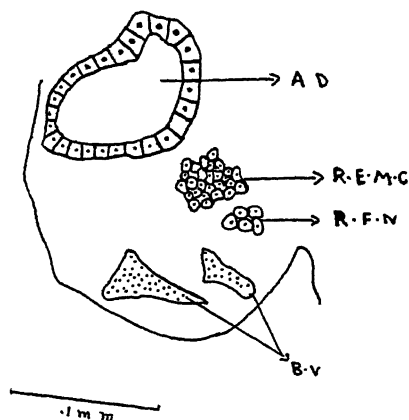


FIG. 1.

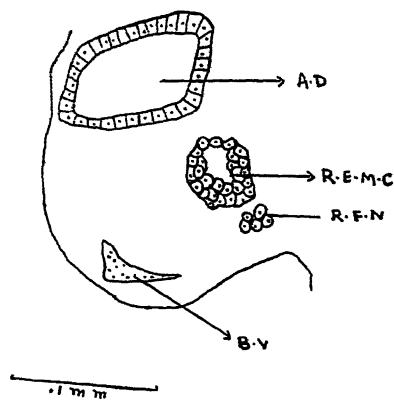


FIG. 2.

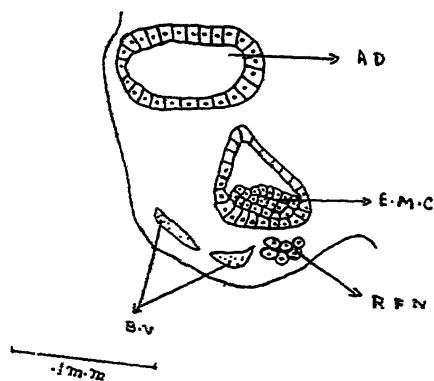


FIG. 3.

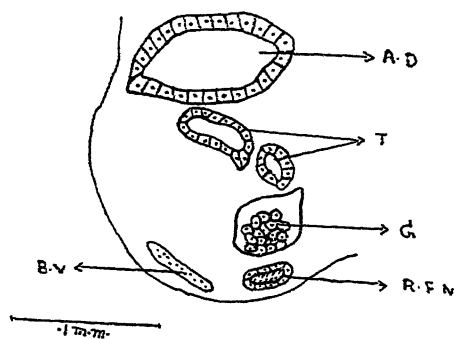


FIG. 4.

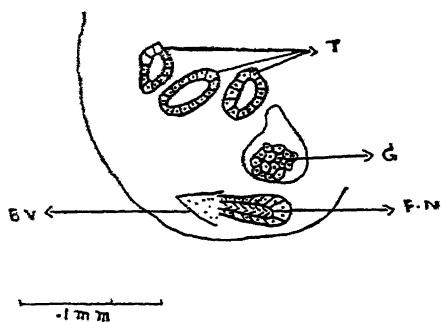


FIG. 5.

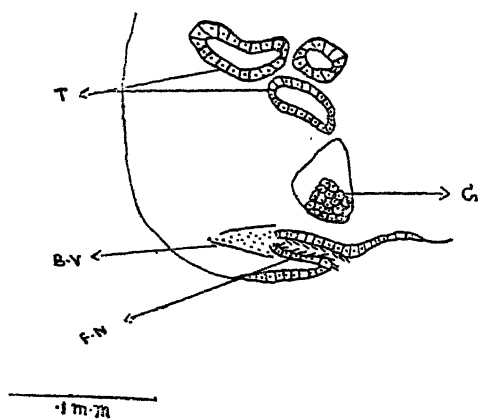


FIG. 6.

*Stage 4.*—Now the condensations of blastema cells representing the rudiments of the straight tubules begin to undergo development. Each condensation becomes a straight tubule. Its proximal end is pressed against the archinephric duct and finally its lumen becomes continuous with that of the archinephric duct (St. Fig. 6 and 7). Each straight tubule as it grows gives rise to five to six outgrowths or secondary tubules which extend ventrally (O.St. Figs. 9, 10, 11 and 14). The one which is proximal to the archinephric duct is the first formed secondary tubule and the distal one is the last formed one.

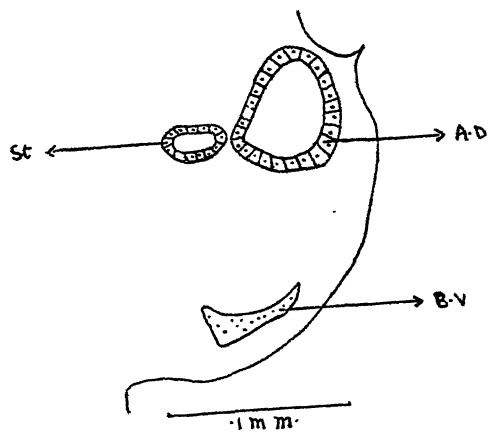


FIG. 7.

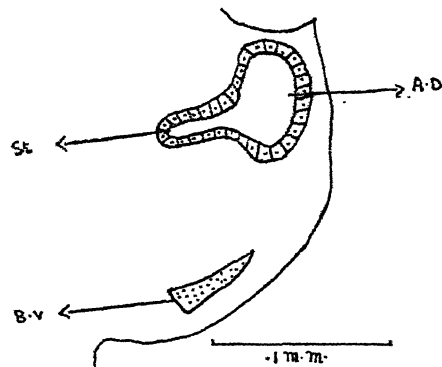


FIG. 8.

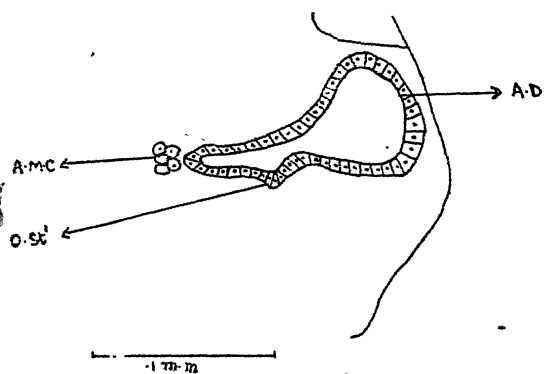


FIG. 9.

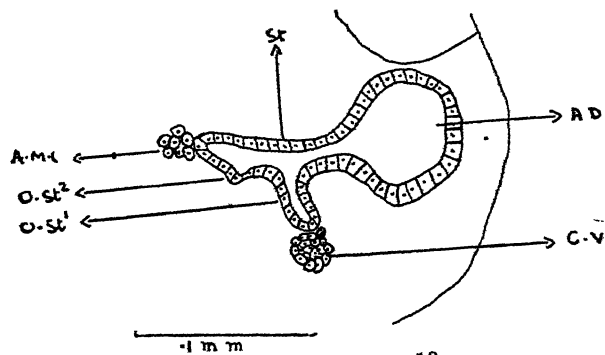


FIG. 10.

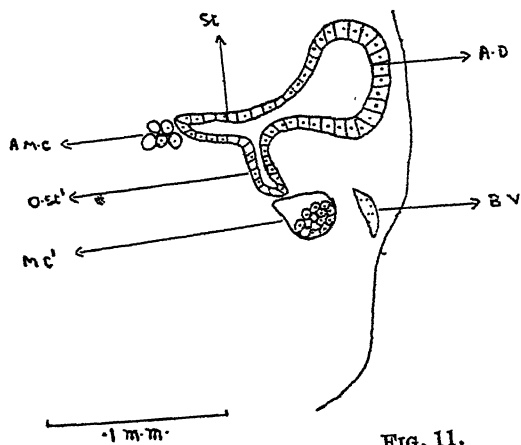


FIG. 11.

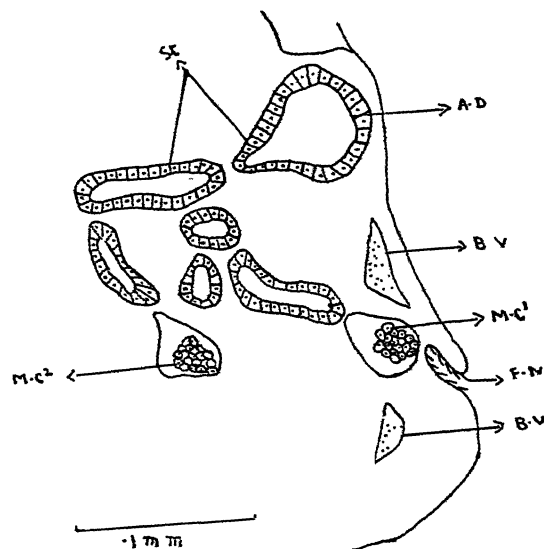


FIG. 12.

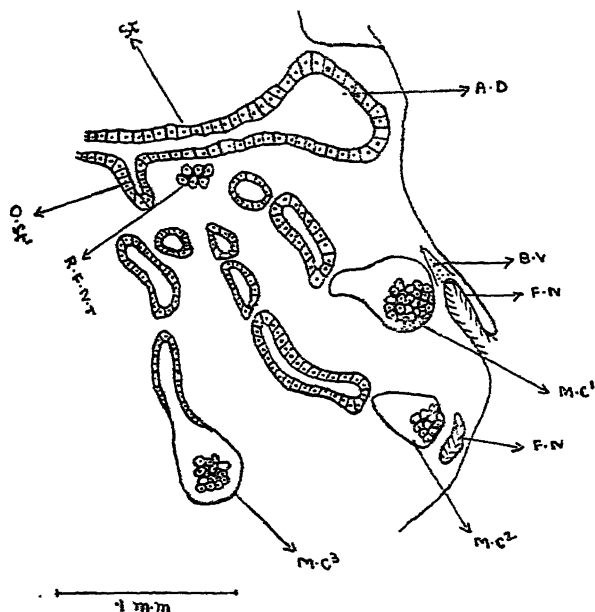


FIG. 13.

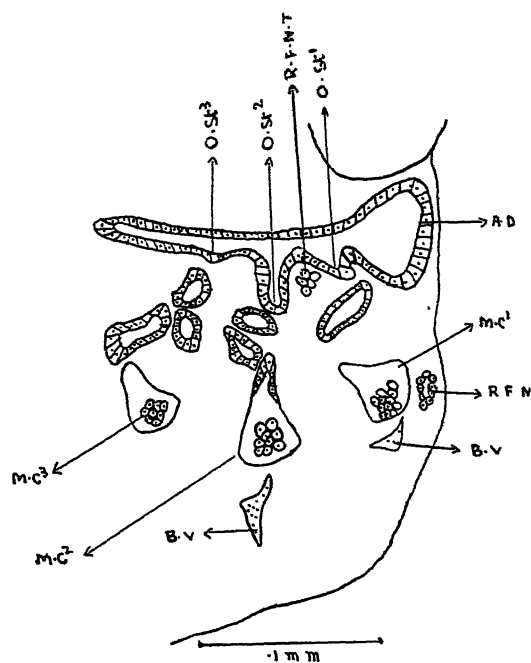


FIG. 14.

Now at the free end of each secondary tubule is a condensation of blastema cells which are loosely packed like those of the nephroblast vesicles (C.V. Fig. 10). These condensations are the capsuloblast vesicles which develop into the later malpighian capsules. The number of capsuloblast vesicles formed in connection with each straight tubule is equal to the number of secondary tubules.

In addition to these condensations of capsuloblast vesicles at the free ends of the secondary tubules, there is another condensation of blastema cells at the free end of the straight tubule itself (A.M.C. Figs. 10 and 11). This does not develop into a malpighian capsule and is the abortive malpighian capsule already met with in *Rana* and *Rhacophorus*.

Each capsuloblast vesicle when it is first condensed is spherical. Later by a reorientation of the cells it becomes pear-shaped (Fig. 11). The narrow end of the pear at which the stalk of the pear is to be inserted abuts against the free end of the secondary tubule. Further reorientation takes place with the result that a malpighian capsule with its glomerulus is formed. As the corresponding secondary tubule grows downwards its free end gets connected with the narrow end of the pear-shaped malpighian capsule. The cavity of the malpighian capsule becomes continuous with the lumen of the

secondary tubule and thus indirectly with the lumen of the archinephric duct. With the further growth of the secondary tubule the malpighian capsule is carried towards the peritoneal wall (Figs. 12 and 13).

Then the next capsuloblast vesicle develops into a malpighian capsule and is pushed to the peritoneal wall by its corresponding secondary tubule. This second capsule comes to occupy a position below that of the first malpighian capsule (Fig. 13). Thus the later malpighian capsules are all pushed towards the peritoneal wall in regular order so that the first formed capsule is at the top while the last formed one occupies the lowest position.

With the approach of each malpighian capsule towards the peritoneal wall a small condensation of cells (R.F.N. Fig. 14) appears in between the capsule and the peritoneal wall. This is the rudiment of the later peritoneal funnel developed in connection with the later malpighian capsule. This develops on exactly the same lines as the early peritoneal funnel and establishes a connection between the blood circulation and the coelom (Figs. 13, 14 and 15). These rudiments of the later peritoneal funnels are never noticed when the malpighian capsules are away from the peritoneal wall. As in the case of *Rhacophorus* the condensation of the rudiment makes its first

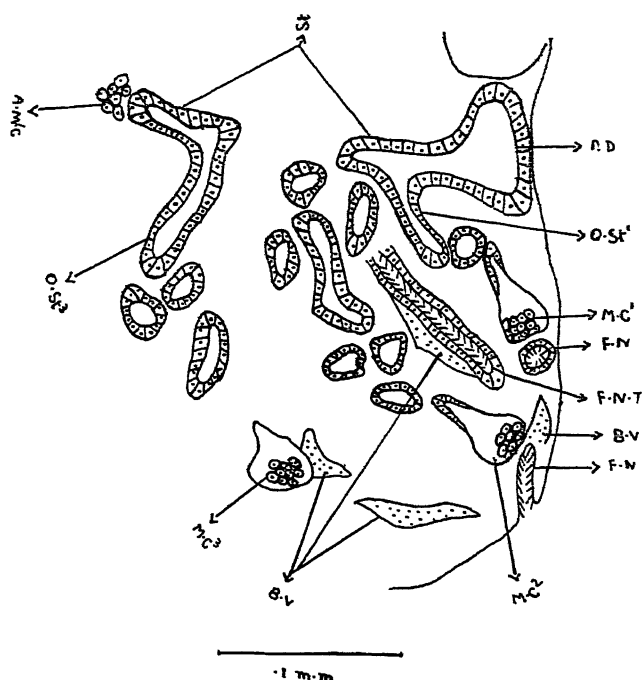


FIG. 15.

appearance only with the approach of the malpighian capsule near the peritoneal wall.

*Stages 5 and 6.*—The early malpighian capsules and their glomeruli begin to degenerate and disappear. Both in longitudinal and transverse sections degenerating tubules and malpighian capsules can be noticed. In no case a degenerating peritoneal funnel could be detected. As in *Rhacophorus* the early peritoneal funnels are evidently retained.

At this stage the mesonephros is marked by the presence of a large number of peritoneal funnels which far outnumber the later malpighian capsules. As in the case of *Rana* and *Rhacophorus* they are produced by the funnel-forming tubule (R.F.N.T. and F.N.T. Figs. 13, 14 and 15). Its origin and subsequent development are on exactly the same lines. The number of funnels produced in each tubule is difficult to determine. The cilia within the lumen of the tubule are not confined to the lower third of the tubule. They extend farther upwards and in some cases almost the entire tubule is ciliated inwards.

#### *Discussion.*

A study of the development of the mesonephros of *Rana* (Gray, 1930), *Rhacophorus* (Reddy, 1938) and *Bufo* shows a very close similarity. There are however some differences. But these are of minor importance and are easily explained with reference to a general line of development typical of Anura.

In all the three cases the early malpighian capsules, their glomeruli and their attendant tubules arise from nephroblast vesicles. But the origin of the rudiment of the early peritoneal funnels is not alike. In *Rana* it arises from a condensation of blastema cells in front of the capsule after the latter has been perfected. In *Bufo* this condensation appears very early almost simultaneous with the condensation of the nephroblast vesicle. In both cases the rudiment arising from a condensation of the blastema cells is quite distinct and separate from the condensation of the nephroblast vesicle. In *Bufo* though it appears early it begins to develop only after the formation of the malpighian capsule has been completed.

The case is very different in *Rhacophorus*. The rudiment arises as a thickening of the outer wall of the early malpighian capsule. This thickening severs its connections with the capsule and then develops into a peritoneal funnel.

This apparently wide deviation is easily brought in a line with the developments of the funnels in other forms if we assume that in this case the early condensation of the blastema cells is a compound one, *i.e.*, it consists

of both the condensation of the nephroblast vesicles and the condensation of the rudiment of the peritoneal funnels. In *Rana* and *Bufo* the two condensations are separate. While in *Rana* the condensation of the early peritoneal funnel appears long after the condensation of the nephroblast vesicle, in *Bufo* both appear simultaneously. From that of *Bufo* the condition in *Rhacophorus* is but a slight step. The two condensations are not only simultaneous but also identical. From this common condensation the rudiment of the early peritoneal funnel gets differentiated at a later stage as a thickening which subsequently gets detached and develops like the rudiments of its kind in *Rana* and *Bufo*.

In Urodela (*Triton*; Gray, 1932) the early peritoneal funnel arises in a manner similar to that of *Rhacophorus*. The malpighian capsule and the funnel arrive from a common condensation. But the rudiment of the funnel never gets separated from the rudiment of the malpighian capsule. When both of them get perfected the cavity of the malpighian capsule is kept in direct communication with the coelom by the peritoneal funnel.

With the appearance of later malpighian units the early malpighian capsules with their glomeruli and tubules disappear. The early peritoneal funnels in *Rhacophorus* and *Bufo* persist.

The formation of the later malpighian units in all the three types is alike excepting for the development of the capsuloblast vesicles in *Bufo*. In all cases the rudiments of the straight tubules and the secondary outgrowths appear as condensations of blastema cells along the dorso-median wall of the archinephric duct.

In the case of *Rana* and *Rhacophorus* the blastema extends in the form of dorso-ventrally extending tracts. In each tract a string of three to four condensations or capsuloblast vesicles appear. Each condensation develops into a later malpighian capsule and sends upwards a tubule to meet the downwardly growing corresponding outgrowth of the straight tubule.

In *Bufo* the case is different. The blastema is not in the form of dorso-ventrally extending tracts but has irregular distribution. At the tip of each outgrowth of the straight tubule a capsuloblast vesicle is condensed. Each condensation when it is perfected gives rise to a pear-shaped malpighian capsule, the narrow end of which presses against the free tip of the outgrowth of the straight tubule. Unlike the previous cases there is no tubule developing from the malpighian capsule to establish connection with the secondary tubule. The very close proximity of the condensation of the capsuloblast vesicle to the secondary tubule does not necessitate the development of this tubule which is so essential to establish a communication between the

archinephric duct and the malpighian capsule in *Rana* and *Rhacophorus*. The narrow end of the malpighian capsule, which abuts against the free end of the secondary tubule in *Bufo*, is comparable to the entire tubule of the later malpighian capsule in *Rana* and *Rhacophorus*.

The rudiments of the peritoneal funnels developed in connection with the later malpighian capsules invariably arise from condensations of blastema cells in front of the capsules. In *Rana* these condensations arise in front of the malpighian capsules long before the latter reach the peritoneal wall while in *Bufo* and *Rhacophorus* they appear with the approach of the malpighian capsules towards the peritoneal wall.

In all the three types the formation and the function of the funnel-forming tubule are alike.

From the above survey of the development of the mesonephros in *Rana*, *Rhacophorus* and *Bufo* the following may be formulated as the general line of formation of the mesonephros typical of Anura.

The mesonephric units in Anura arise from condensations of blastema cells.

The units appear in two sets. The early set is characterised by as many peritoneal funnels as there are malpighian capsules, while the latter set is characterised by peritoneal funnels which outnumber the capsules.

The early malpighian capsules have a direct communication with the archinephric duct. They degenerate and disappear with the advent of the later units.

The rudiments of early peritoneal funnels arise from condensations of blastema cells. The condensation might appear later on after the malpighian capsule is formed or it might appear simultaneously with the nephroblast vesicle or it might appear combined with the condensation of the nephroblast vesicle from which it might get differentiated and detached subsequently. The early peritoneal funnels establish a direct communication between blood circulation and coelom. These do not degenerate.

Later malpighian capsules appear as capsuloblast condensations and are connected indirectly with the archinephric duct by the straight tubules and their outgrowths. The connection between the outgrowths of the straight tubule and the cavity of the malpighian capsules might be brought about by the latter sending upwards tubules to join the downwardly growing outgrowths or by the outgrowths forcing themselves into the capsules when the latter are formed in close proximity.

The later peritoneal funnels arise in two ways. (1) They arise from condensations of blastema cells between the later malpighian capsules and

the peritoneal wall. (2) They are produced by a special funnel-forming tubule. Like the early peritoneal funnels they establish a direct connection between the coelom and the blood circulation.

*Summary.*

1. The general development of the mesonephros in *Bufo* is similar to that of *Rana* (Gray, 1930) and *Rhacophorus* (Reddy, 1938).

2. Each malpighian unit arises from a nephroblast vesicle which is a condensation of 14 to 16 blastema cells.

3. 10 to 13 nephroblast vesicles are developed.

4. The rudiment of the early peritoneal funnel appears as condensation of 4 to 6 blastema cells in front of the nephroblast vesicles.

5. The condensation is simultaneous with that of the nephroblast vesicle.

6. The rudiment develops after the perfection of the early malpighian capsule and establishes a connection between the coelom and blood circulation.

7. The early peritoneal funnels persist while early malpighian capsules with their tubules and glomeruli degenerate and disappear.

8. The straight tubules and their outgrowths arise from 13 to 15 condensations on the dorsomedian wall of the archinephric duct.

9. At the free end of the tubule is an abortive malpighian capsule.

10. At the free end of each outgrowth or secondary tubule is a condensation of capsuloblast vesicle.

11. A later malpighian capsule arises from each capsuloblast vesicle.

12. Five to six capsuloblast vesicles are developed.

13. The malpighian capsules do not develop any upwardly running tubules.

14. Each outgrowth of the straight tubules gets connected with the malpighian capsule, so that the lumen of the tubule is continuous with the cavity of the capsule.

15. The peritoneal funnels are developed from condensation of blastema cells which appear when the malpighian capsules approach the peritoneal wall.

16. The later peritoneal funnels also establish a connection between the coelom and blood circulation.

17. The rudiment of the funnel-forming tubule arises from a condensation of cells near the straight tubule.

18. Greater portion of the lumen of the tubule is ciliated.
19. The tubule never gets connected with the archinephric duct either directly or indirectly.
20. By a process of repeated constrictions, it gives rise to a number of peritoneal funnels ; which also communicate with the coelom and the blood circulation.
21. The differences in the development of the mesonephros in *Rana* and *Rhacophorus* and *Bufo* are explained with reference to general line of development typical of Anura.

#### Acknowledgements.

I have great pleasure in expressing my gratitude to Prof. S. G. M. Ramanujam of the Presidency College, Madras, for his kind interest and to Dr. Peter Gray of the Edinburgh University who was kind enough to go through the manuscript.

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# EXPLANATION OF FIGURES.

- FIGS. 1 to 6      Showing the various stages in the development of the early peritoneal funnel.
- FIGS. 7 & 8      Showing the development of the straight tubule.
- FIG. 9            Showing the development of an outgrowth from the straight tubule and the rudiment of the abortive malpighian capsule.
- FIGS. 10 & 11    Showing the formation of the first capsuloblast vesicle.
- FIGS. 12 to 14    Showing the formation of a row of later malpighian capsules.
- FIG. 15           Showing the later malpighian capsules near the peritoneal wall and the formation of the later peritoneal funnels.

## REFERENCE LETTERS.

<i>A.D.</i>	.. Archinephric duct.
<i>A.M.C.</i>	.. Abortive malpighian capsule.
<i>B.</i>	.. Blastema.
<i>B.V.</i>	.. Blood vessel.
<i>C.F.N.</i>	.. Point at which the peritoneal funnel is constricted off.
<i>D.G.</i>	.. Degenerating malpighian glomerules.
<i>E.C.</i>	.. Capsule of the early malpighian unit in which the glomerulus has completely disappeared.
<i>E.M.C.</i>	.. Early malpighian capsule.
<i>F.N.</i>	.. Peritoneal funnel.
<i>F.N.<sup>1</sup> &amp; F.N.<sup>2</sup></i>	.. Peritoneal funnels produced from funnel-forming tubule.
<i>F.N.T.</i>	.. Funnel-forming tubule.
<i>G.</i>	.. Glomerulus.
<i>I.N.</i>	.. Opening of the peritoneal funnel into the blood vessel.
<i>M.C.<sup>1</sup>, M.C.<sup>2</sup> &amp; M.C.<sup>3</sup></i>	.. Later malpighian capsules developed from capsuloblast vesicles.
<i>O.N.</i>	.. Opening of the peritoneal funnel into the coelom.
<i>O.St.<sup>1</sup> &amp; O.St.<sup>2</sup></i>	.. Outgrowths of the straight tubule.
<i>P.W.</i>	.. Peritoneal wall.
<i>R.F.N.</i>	.. Rudiment of the peritoneal funnel.
<i>R.F.N.T.</i>	.. Rudiment of the funnel-forming tubule.
<i>R.M.G.</i>	.. Rudiment of the early malpighian glomerulus.
<i>R.St.</i>	.. Rudiment of the straight tubule.
<i>St.</i>	.. Straight tubule.
<i>T.</i>	.. Tubules.

## NOTES ON INDIAN HEPATICS.

### II. Sikkim Himalayas and Bengal.

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Received October 19, 1938.

[Communicated by Dr. H. Chaudhuri, D.Sc. (Lond.), Ph.D., D.I.C.]

PRIOR to the publication of "Liverworts of the Western Himalayas and the Punjab Plains" much more was known about the Hepatic Flora of Sikkim as compared with any other botanical area. There are two reasons for this. This area on account of its close proximity to Calcutta—the headquarters of the Botanical Survey of India—has attracted the attention of Hooker, Thompson, Wallich and others. Secondly, the clergy stationed at Kurseong have made valuable collections and passed them on to the workers in Europe. In spite of this, however, an examination of more recent collections shows some new species and a number of new records. So it seems necessary to publish these results.

Material for this note has been provided by the following collections :

- (i) By S. J. Kurz in 1867–68, made available by the courtesy of Dr. K. P. Biswas.
- (ii) By Rev. Bretaudeau in 1895, made available by the courtesy of Mr. C. E. Parkinson.
- (iii) An extensive collection from Sikkim by late Prof. S. R. Kashyap in the summers of 1929 and 1930.
- (iv) An intensive collection from Darjeeling and its suburbs by the writer in the summer of 1935.

Thanks are due to those who collected material for this note, to Dr. K. P. Biswas and Mr. C. E. Parkinson for making their herbarium specimens available to the writer, to Dr. Fr. Verdoorn for the determination of some Lejeuneace and to Mr. W. E. Nicholson for comparison and determination of some specimens of other genera.

#### RICCIACEÆ.

##### *Riccia* L.

*R. himalayensis* St. (1 : 93).

Calcutta, Botanical gardens, 1929, 6 ;  
Darjeeling, Tirrunarayanan, 1917, 7 ;  
Temi, 945.

## MARCHANTIACEÆ.

*Targionia* L.

*T. hypophylla* L. (1 : 57).

Darjeeling, common, 116, 631, 950, 2103 ;  
Ghoom, 680, 683.

*Cyathodium* Kunze

*C. tuberosum* Kash. (1 : 53).

Darjeeling, common, 632, 920, 1694 ;  
Rajmahal, J. Kurz, 1868, K., 1798.

*Plagiochasma* L. et L.

*P. appendiculatum* L. et L. (1 : 76).

Darjeeling, common, 921, 2106.

*P. articulatum* Kash. (1 : 75).

Darjeeling, common, 659, 2106 ;  
Gyanste, 931.

*Reboulia* Raddi

*R. hemispherica* (L.) Raddi (1 : 72).

Darjeeling, common, 661, 607, 1689, 2104 ;  
Kurseong, Kurz, 1868, K. 2507.

*Fimbriaria* Nees

*F. angusta* St. (1 : 63).

Darjeeling, common, 633, 2099.

*F. blumena* Nees (1 : 62).

Darjeeling, common, 634, 677, 1700, 2100.

*F. leptophylla* Mont. (4 : 100).

Phallot, J. Kurz, 1868, K. 2222 ;  
Locality not noted, J. Kurz, 1868, K. 2050.

*F. mussuriensis* Kash. (1 : 64).

Darjeeling, common, 635, 679, 953, 1696, 2001.

*F. reticulata* Kash. (1 : 65).

Darjeeling, 1684, 2162.

*Conocephalum* Necker

*C. conicum* (L.) Necker (1 : 44).

Darjeeling, common, 616, 636, 1022, 1684, 1697, 2109.

*Lunularia* Mich.

*L. cruciata* Dum. (1 : 46).

Darjeeling, Tirrunarayanan, Bh. 18 (1).

*Dumortiera*, R. Bl. et Nees*D. hirsuta* R. Bl. et Nees (1 : 42).

Darjeeling, common, 617, 637, 631, 1685, 2109.

*Wiesnerella* Schiff.*W. denudata* (Mitt.) St. (1 : 40).

Darjeeling, Bh. 22 (1 c).

*Marchantia* L.*M. nepalensis* L. et L. (1 : 36).

Darjeeling, common, 611, 612, 615, 618, 619, 639, 674, 1695, 2109.

*M. nitida* L. et L. (4 : 184).

Sikkim, J. Kurz.

*M. palmata* Nees (1 : 34).

Darjeeling, common, 640, 2108 ;

Sikkim, locality not noted, Kurz, 1868, K. 1760 ;

Kurseong, Kurz, 1868, K. 2189.

Calcutta, Kurz, 1868, K. 2533.

*M. polymorpha* L. (1 : 32).

Darjeeling, common, 638, 948, 2110, 2111.

*M. subintegra* Mitt. (4 : 178).

Darjeeling, common, 2102.

## ANEURACEÆ.

*Aneura* Dum.*A. levieri* Dum Schiffn. (1 : 113).

Darjeeling, very common, 644, 705, 2113.

*Metzgeria* Raddi*M. hamata* Lindb. (4 : 297).

Darjeeling, 754.

*M. himalayensis* Kash. (2 : 111).

Darjeeling, 687, 793.

*M. longitexta* St. (4 : 288).

Sinchul, Kurz, 1868, K. 2357.

## CODONIACEÆ.

*Calycularia* Mitt.*C. crispula* Mitt. (2 : 103).

Darjeeling, common, 620, 621, 647,

*Pellia* Raddi

*P. calycina* (Tayl.) Nees (2 : 100).

Darjeeling, 2115.

*P. epiphylla* (L.) Lindb. (2 : 101).

Darjeeling, Bh. 37 (2), Bh. 37 (2 a).

*Fossombronina* Raddi

*F. himalayensis* Kash. (1 : 99).

Darjeeling, common, 649, 651, 678, 2116.

## LOPHOZIACEÆ.

*Notoscyphus* Mitt.

*N. paroicus* Schiffn. (5 : 37).

Darjeeling, 877, 889.

*Alicularia* Corda

*A. scalaris* (Schrad.) Corda (3 : 126).

Darjeeling, 728.

*Eucalyx* Breidl.

*E. hyalinus* (Lyell) Breidl. (3 : 138).

Punkabaree (2-4,000), J. Kurz, 1868, K. 2440 and K. 2450.

*Aplozia* Dum.

*A. cæspitica* (Lindb.) Dum. (3 : 143).

Darjeeling, common, 2169 ;

Rajmahal Hills, Kurz. 1868, K. 1799.

*A. crenulata* (Sm.) Dum. (3 : 141).

Darjeeling, common, 713, 723.

*A. purpurata* (Mitt.) Chopra, (5 : 51).

Darjeeling, common, 1711, 1712 ;

Sariong, Kurz, 1868, K. 2363.

*A. riparia* (Tayl.) Dum. (3 : 147).

Sinchul, J. Kurz, 1868, K. 2376.

Darjeeling, 1929, 1020, 1026.

*A. sanguinolenta* (Griffth.) Chopra (5 : 51).

Darjeeling, 697, 2170.

*A. sphærocarpa* (Hook.) Dum. (3 : 144).

Darjeeling, common, 703, 710.

*Anastrophyllum* (Spruce) St.

*A. donianum* (Hook.) St. (3 : 159).

Darjeeling, 704, 708, 722.

*A.* sp. probably *A. cucullifolium* St. (9 : 104).

Phallot, J. Kurz, 1868, K. 2247.

*Cuspidatula* St.

*Cuspidatula nicholsonii* Chopra sp. nov.

Sterile, medium red to purple, densely caespitose on rocks. Stem up to 3 cm. long, simple or sparsely branched, ascending, occasionally with subfloral innovations. Rhizoids colourless, confined to old parts. Leaves ovate, semi-amplexicaul, almost transversely inserted, base decurrent on both sides, conduplicate-concave, upper half often reflexed, apex acute or acuminate; apical cells  $18\mu$ , basal cells  $54\mu \times 18\mu$ ; walls thick, trigones thick, nodulose. Bracts like the leaves, entire or bilobed. Perianth leaf exerted, cylindrical oblong, plicate, mouth laciniate. Rest not seen.

*Hab.*—On rocks.

*Distrib.*—Darjeeling 701, 704, 731.

*Note.*—As a token of appreciation for the kind and ungrudging help given to me by Mr. W. E. Nicholson of Lewes, England, I have named this plant after him.

This species shows some interesting variations. In some plants the leaves are bilobed like a typical *Anastrophyllum*, while other leaves are faintly bilobed, and still others are typically acute or acuminate. In other plants with acute or acuminate leaves a leaf may be met with showing slight traces of a notch at the apex. The bracts may be acute or bilobed. On one stem one bract may be acute and the other bilobed. Thus this species is a link between *Anastrophyllum* where the leaves are bilobed and bracts are acute and *Cuspidatula* where the leaves are acute and bracts bilobed and dentate.

*Lophozia* Dum.

*L.* sp. inter affinis *L. turbinata* and *L. badensis*,

Darjeeling, 714.

*L.* sp. inter affinis *L. bidentata* and *L. guttulata*,

Kurseong, J. Kurz, 1868, K. 2501.

*Acrobolbus* Nees

*A.* sp. Phallot (12–13,000), J. Kurz, 1868, K. 2202.

*Syzygiella* Spruce*Syzygiella dentata* Chopra sp. nov.

Sterile, medium, robust, deep-brown, dense depresso-caespitose, on bark. Stem up to 6 cm. long, simple or sparsely pinnate. Leaves opposite, imbricate, subsecund, triangular-ovate, base narrow, antically decurrent, postically amplicate, non-decurrent, antical margin unarmed, recurved, postical margin and apex with several strong divergent teeth. Apical cells  $12-20\ \mu$ ; vitta cells  $50-58\ \mu \times 18-24\ \mu$ ; walls and trigones equally thickened throughout. Rest not seen.

*Hab.*—On bark.

*Distrib.*—Sinchal, J. Kurz, 1868, K. 2391.

*Anastrepta* (Lindb.) Schiffn.

*A. orcadensis* (Hook.) Schiffn. (3 : 220).

Sikkim Himalayas, J. Kurz, K. 2110.

*A. sikkimensis* St. (9 : 119).

Darjeeling, 927.

*Leptoscyphus* Mitt.

*L. Taylora* (Hook.) Mitt. (3 : 235).

Darjeeling, 742, 855.

*Lophocolea* Dum.

*L. cuspidata* Limpr. (3 : 241).

Darjeeling, 800, 907.

*L. bidentata* (L.) Dum. (2 : 66).

Darjeeling, 2171.

*Chiloscyphus* Corda

*C. argutus* Nees (2 : 62).

Darjeeling, 846.

*C. coalitus* (Hook.) Dum. (6 : 242).

Darjeeling, 944.

*C. flaccidus* (Mitt.) St. (6 : 210).

Darjeeling, 687, 764.

*C. Gammianus* St. (6 : 217).

Kurseong, Bretaudeau, 1895, Her. F.R.I., Dehra Dun, under the name *Plagiochila chiloscyphoids* St.

*C. inflatus* St. (2 : 61).

Darjeeling, 1010.

*C. perfoliatus* (Mont.) Nees (6 : 204).

Darjeeling, 2172.

*C. polyanthos* (L.) Corda (2 : 63).

Darjeeling, 681, 685 ;

Phallot, J. Kurz, 1868, K. 2320.

*Saccogyna* Dum.

*S. subalternifolia* St. (9 : 317).

Darjeeling, 2173.

## CEPHALOZIACEÆ.

*Schiffneria* St.

*S. viridis* St. (10 : part 1, 55).

Darjeeling, rare, 710, 720.

*Cephaloxia* Dum.

*C. Gollani* St. (2 : 59).

Darjeeling, 2174.

*Nowellia* Mitt.

*Nowellia orientalis* Chopra sp. nov.

Sterile, brown, mixed with other hepatics, on rocks. Stem 1–3 cm. long, pellucid, branched. Rhizoids scarce, leaves imbricate, concave, almost transversely inserted by a narrow non-decurrent base, obliquely ovate, antical margin slightly curved, postical flat with a sacculate base, shortly bilobed, lobes coarsely dentate. Cells hexagonal, walls thick, trigones acute ; median cells 20–25  $\mu$ . Rest not seen.

*Hab.*—On rocks.

*Distrib.*—Darjeeling, 743, 745.

*Note.*—The species is distinct because none of the three known species has so distinctly toothed leaves. These plants superficially resemble *Anastrophyllum piligerum* (Nees) Spr. but can easily be distinguished by the sacculate base.

*Odontoschisma* Dum.

*O. sphagni* (Dicks.) Dum. (3 : 305).

Phallot, J. Kurz, 1868, 2202.

*Calypogeia* Raddi

*C. fissa* (L.) Raddi (3 : 318)

Darjeeling, sent by P. Maheshwari, Bh. 91 (1).

*C. Hartlessiana* St. (6 : 399).

Darjeeling, 2175.

*C. lunata* Mitt. (6 : 401).

Darjeeling, 770, 779.

*C. renistipula* St. (2 : 58).

Darjeeling, common, 2176.

Leaves very variable, usually obtuse, but may be truncate, bidentate, or acute even.

*C. trichomanis* (L.) Corda (3 : 316).

Darjeeling, 727.

*C. sp.*

Darjeeling, 711, 722, 725.

*Mastigobryum* Nees

(*Bazzania* S.F. Gray).

*M. appendiculatum* Mitt. (6 : 498).

Kurseong, Bretaudeau, Oct. 1895 ; Herb. F.R.I., Dehra Dun.

*M. decurvum* Nees (6 : 513).

Sikkim Himalayas, J. Kurz, K. 2110.

*M. Gammianum* St. (6 : 461).

Darjeeling, 2177 ;

Stephani (*Sp. Hep.* Vol. III, p. 416 and p. 514) has described two different plants under the same specific name *M. Gammianum* St. A comparison based on the description of our plants with those of Stephani shows that our specimens are either *M. Gammianum* St. p. 461 or near it.

*M. himalayannum* Mitt. (6 : 444).

Phallot, J. Kurz, 1868, K. 2336 ;

Sariong, J. Kurz, 1868, K. 2420.

*M. oblongum* Mitt. (6 : 464).

Darjeeling, 2178 ;

Kurseong, Bretaudeau, 1895, Herb. F.R.I., Dehra Dun.

*M. sikkimensis* St. (6 : 434).

Darjeeling, 2179.

*Lepidozia* Dum.

*L. brevifolia* Mitt. (6 : 624).

Darjeeling, 805, 806.

*L. himalayensis* St. (6 : 617).

Darjeeling, 702.

*L. macrocalyx* St. (6 : 623).

Darjeeling, 698, 722 ;

Sikkim, Thomson, Herb. F.R.I., Dehra Dun.

*L. robusta* St. (6 : 613).

Jallaphar (Darjeeling), J. Kurz, 1868, K. 2405.

*L. stahlii* St. (6 : 616).

Darjeeling, 696.

*L. tridens* St. (6 : 616).

Darjeeling, 717, 876.

#### PTILIDIACEÆ.

##### *Blepharostoma* Dum.

*B. trichophyllum* (L.) Dum. (2 : 53).

Darjeeling, common, 697, 706.

##### *Chandoanthus* Mitt.

*C. hirtellus* (Weber) Mitt. (6 : 643).

Darjeeling, common, 747 ;

Kurseong, Bretaudeau, 1895, Herb. F.R.I., Dehra Dun.

Phallot, J. Kurz, 1868, K. 2260, K. 2328.

##### *Herberta* S. F. Gray

(= *Schisma* Dum.)

*H. chinensis* (St.) (7 : 26).

Darjeeling, common, 708, 724 ;

Phallot, J. Kurz, 1868, K. 2202, 2247, 2314.

*H. dicranum* (Tayl.) Trevis, (7 : 24).

Darjeeling, common, 725, 728 ;

Phallot, J. Kurz, 1868, K. 2225.

*H. Fleischerii* (St.) (9 : 358).

Phallot, J. Kurz, 1868, K. 2225.

*H. longifissum* (St.) (7 : 27).

Kurseong, Bretaudeau, 1895, Herb. F.R.I., Dehra Dun.

*H. pinnatum* (St.) (9 : 361).

Sinchul, J. Kurz, 1868, K. 2368 ;

Sikkim, J. Kurz, 1868, K. 2142.

*H. wichuræ* (St.) (7 : 25).

Darjeeling, 711.

## SCAPANIACEÆ.

*Diplophyllum* Dum.

*D. contortum* (Mitt.) St. (7 : 116).

Darjeeling, 697, 926.

*D. ferrugineum* (L. et L.) St. (7 : 115).

Darjeeling, common, 693, 694, 717.

*D. orientalis* St. (2 : 47).

Darjeeling, 715.

*D. nepalense* (Nees) St. (7 : 116).

Darjeeling, 736, 827.

*Scapania* Dum.

*S. Griffithii* Schiffn. (7 : 141).

Darjeeling, 865.

*S. Handelii* Nicholson (11 : 19).

Darjeeling, 743.

*S. levierio* C. Mull. (7 : 139).

Darjeeling, 768, 791.

*S. planifolia* (Hook.) Dum. (3 : 381).

Darjeeling, 705, 1010.

*S. secunda*, St. (7 : 144).

Darjeeling, 704, 725.

## RADULACEÆ.

*Radula* Dum.

*R. complanata* (L.) Dum. (2 : 44).

Darjeeling, C. 896.

*R. Lindbergiana* G. (2 : 46).

Darjeeling, 822.

## MADOTHECACEÆ.

*Madotheca* Dum.

*M. campylophylla* L. et L. (2 : 29).

Darjeeling, 2195.

*M. perrottetiana* Mont. (7 : 307).

Darjeeling, 2193, 2194.

*M. ptychantha* Mitt. (7 : 302).

Darjeeling, 2192.

## LEJEUNEACEÆ.

*Frullania* Raddi

(Fr. Verdoorn determination.)

*F. Grevilleana* (Tayl.) Nees (2 : 16).

Darjeeling, 706, 759, 772.

*F. Grevilleana* var. *densa* Schiffn.

Darjeeling, 901.

*F. musicola* St. (2 : 14).

Darjeeling, 798.

*F. nepalensis* L. et L. (7 : 452).

Darjeeling, very common, 718, 745, 757, 801.

*F. physantha* Mitt. (7 : 353).

Darjeeling, 808, 837, 888.

*F. squarrosa* Nees (2 : 11).

Darjeeling, 752.

(Stephani determination.)

*F. evoluta* Mitt. (7 : 451, 557).

Kurseong, Bretaudeau, 1894, Herb. F.R.I., Dehra Dun.

*F. Wallichiana* Mitt. (7 : 348).

Kurseong, Bretaudeau, 1894, Herb. F.R.I., Dehra Dun.

(Chopra determination.)

*F. orbicularis* Aust. (7 : 557).

Sinchul, J. Kurz, 1868, K. 2393 ;

Phallot, J. Kurz, 1868, K. 2352.

*F. retusa* Mitt. (2 : 13).

Phallot, J. Kurz, 1868, K. 2227 ;

Punkabaree, J. Kurz, 1868, K. 2473.

Darjeeling, 2196.

*F. squarrosa* Nees (2 : 11).

Punkabaree, J. Kurz, 1868, K. 2471, K. 2476.

*Ptychanthus* Nees*P. argutus* St. (7 : 745).

Sinchul, J. Kurz, 1868, K. 2389 ;

Sariong, J. Kurz, 1868, K. 2417 ;

Punkabaree, J. Kurz, 1868, K. 2477, K. 2478.

*P. birmensis* St. (7 : 745).

Kurseong, Bretaudeau, Herb. F.R.I., Dehra Dun.

*P. chinensis* St. (2 : 21).

Darjeeling, 823, 828.

*P. Neitneri* St. (7 : 750).

Kurseong, Bretaudeau, Herb. F.R.I., Dehra Dun.

*P. perrottetii* St. (2 : 20).

Darjeeling, 776, 780.

*Lopholejeunea* Schiffner

*L. subfusca* (Nees) St. (8 : 86).

Darjeeling, C 909.

*L. sikkimensis* St. (8 : 87).

Balsun Valley, J. Kurz, 1868, K. 2483.

*Mastigolejeunea* Schiffn.

*M. repleta* (Tayl.) St. (7 : 772).

Balsun Valley, J. Kurz, 1868, K. 2481, K. 2484.

*Marchesinia* Gray

*M. gigantea* St. (8 : 154).

Darjeeling, 854, 903.

*M. sikkimensis* St. (8 : 155)

Darjeeling, 2197.

*Trocholejeunea* Schiffn.

*T. infusata* (Mitt.) Verd (13 : 190)

Syn. 1. *T. levierii* (St.) Schiffn. (12 : 160).

Darjeeling, 847, 864, 866.

2. *B. Levieri* Schiffner (8 : 134).

Punkabaree, J. Kurz, 1868, K. 2474.

*Dicranelejeunea* Schiffner

*D. gliva* St. (8 : 170)

Darjeeling, 887, 892.

*D. sikkimensis* St. (8 : 170).

Darjeeling, 2198.

*Eulejeunea* St.

*L. flava* (Swart.) Nees (3 : 427).

Kurseong, Bretaudeau, Herb. F.R.I., Dehra Dun.

*L. patens* Lindb. (3 : 432).

Goke ridge, J. Kurz, 1868, K. 2194.

*Microlejeunea* St.*M. sp.*

Darjeeling, 858.

## ANTHOCEROTACEÆ.

*Anthoceros* L.*A. erectus* Kash. (1 : 25).

Darjeeling, 622.

*A. himalayensis* Kash. (1 : 26).

Darjeeling, 616.

*Notothyla* Sull.*Notothylas Levieri* Schiffner (1 : 29).

Temi, Sikkim, 946.

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